

SEASONAL GROWTH OF L. SACCHARINA AND L.
DIGITATA : RELATIONS WITH INORGANIC
NUTRIENTS

Nichola Conolly

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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Seasonal Growth of L. saccharina and L. digitata:
Relations with Inorganic Nutrients

A thesis presented for the degree of Doctor of Philosophy
at the
University of St. Andrews
1982
by
Nichola Conolly



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Declaration

I hereby declare that this thesis has been composed by myself, and that it is a record of work which has been done by myself. None of the work has been accepted in any previous application for a degree. Any other sources of information have been acknowledged.

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Nichola Conolly

(ii)

Statement

I, Nichola Conolly, was admitted as a research student of the University of St. Andrews in October 1978 in accordance with Ordinance General No. 12 and the Resolution of the University Court, 1967, No. 1. The thesis was completed in July 1982.

(iii)

Certificate

I hereby declare that Nichola Conolly has been engaged upon research from October 1978 onwards to prepare the accompanying thesis for the degree of Doctor of Philosophy.

Signed

Dr D.C. Weeks

St. Andrews

July 1982

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CHAPTER 1

GENERAL INTRODUCTION

The seasonal pattern of frond growth rate of Laminaria has been known since the early observations of Setchell (1900) but it was not until the introduction of the punched hole technique by Parke (1948) that frond growth (increase in frond length) could be measured accurately. Since then the technique has been used extensively in most Laminaria growth studies.

Parke's (1948) method demonstrated that the seasonally varying growth rates of L. saccharina could be separated into 2 distinct divisions; a 'period of rapid growth' from January to June and one of 'slow growth' from July onwards. More precisely, after a peak in May growth rates decline during the summer and autumn, increasing again only in midwinter (January) with the development of the new frond. The exact timing of the change from slow to rapid growth and vice versa varies with the species of Laminaria investigated and with the location. Such a seasonal pattern necessitates that plants show maximum growth rates in the winter and spring when irradiance and temperature are both lower than in the summer and that growth rates decline during the summer months when irradiance, daylength and seawater temperature are maximal.

The seasonal pattern of linear growth exhibited has several aspects of interest with regard to the factors controlling or effecting a change in growth rate, namely;

the initiation of new frond growth in the winter, the decline in frond growth rates after the May peak and the low growth rates throughout the summer, autumn and early winter.

NEW FROND GROWTH

In L. saccharina and L. digitata new frond growth is first apparent in January, growth rates increasing rapidly after this to the maximum in April/May (Parke, 1948; Cosson, 1967; Perez, 1971). Ambient irradiance is limiting growth during January-April (Luning, 1971; Johnston, Jones & Hunt, 1977) and minimum seawater temperatures are also likely to be limiting growth (Fortes & Luning, 1980) at this time.

The factor(s) responsible for initiating this new frond growth are not immediately apparent. In L. longicruris (Chapman & Craigie, 1977) the nitrate concentration of the seawater is important since the period of rapid growth begins shortly after ambient nitrate levels have increased in the autumn (October/November). Where growth rates of L. saccharina and L. digitata begin to increase 3-4 months after the autumn increase in seawater nitrate levels (see, for example, nutrient surveys of Harvey, 1926; Armstrong, Butler & Boalch, 1972; Foster et al, 1978) a direct causal relationship between nitrate concentration and new frond growth is not so obvious.

It is proposed that there is a photoperiodic control of new frond growth of L. saccharina and L. digitata as the new growth begins only after daylengths increase after

the shortest day in December. Whilst new frond initiation might also be an effect of increasing total irradiance it is doubtful whether an increase in daylength of $\frac{1}{2}$ hour (between December and mid-January) results in a significant increase in total irradiance in situ in midwinter (as indicated in results of Luning & Dring, 1979). This aspect requires further data on in situ underwater irradiances during this period.

Whatever the control involved in initiating new frond development, growth once started increases rapidly despite the low and probably limiting conditions of irradiance and temperature. Light and temperature limitation is clearly overridden by reliance of the new frond on organic reserves supplied by the old lamina (Luning, 1969;1970). The material supplied is mainly mannitol and laminarin accumulated in the old lamina during the previous summer and autumn. There is ample evidence (see review; Kain, 1979) of basipetal translocation enabling these substances to reach the active meristem. The old lamina, therefore, has a supportive role to new frond growth in supplying reserve organic material, but it also adds to the photosynthetic capacity of the plant when there is sufficient irradiance for an assimilatory surplus (Kain, 1979). The relative importance of each contribution is controversial but Luning, Schmitz & Willenbrink (1973) estimated exported carbohydrate reserves from the old frond to be the more important of the 2 roles provided sufficient of the old lamina remains attached for a sufficient time in L. hyperborea.

In L. saccharina and L. longicruris the old lamina may be more important in increasing the photosynthetic area since distal tissue erosion significantly reduces reserves of mannitol and laminarin available for new frond growth in January and February (Johnston et al., 1977; Chapman & Craigie, 1978). Before the stored reserves versus increased photosynthetic area controversy can be resolved further information is required on in situ irradiances during the winter period.

Additional roles of the old frond during new frond growth have received little attention. Possible roles of supplying reserves of N- and P-compounds despite maximum external N and P concentrations and increasing the surface area for mineral nutrient uptake are proposed, based on 3 considerations.

- i Nutrient uptake capacity of the new frond:
some young, actively-dividing tissues (eg. artichoke; Steward & Millar, 1954) show a reduced capacity for mineral nutrient uptake.
- ii Reserves of N and P compounds in the old frond:
tissue N and P content is at maximum levels in the mature tissue during the winter months (Black, 1948).
- iii Basipetal translocation of N and P compounds:
there is evidence for translocation of N-compounds (mainly as amino acids; Schmitz, Luning & Willenbrink, 1972) and P-compounds (Floc'h & Penot, 1971) in Laminaria spp.

DECLINE IN LAMINARIA GROWTH RATES AFTER THE MAY MAXIMUM

The spring decline and low summer growth rates of Laminaria have not been adequately explained by temperature and light controls. Reduced growth during the summer when temperature, photoperiod and irradiance are all higher than in the spring has usually been interpreted by the Harder:Knip hypothesis (Knip, 1914; Harder, 1915; Gessner, 1955) suggesting that the increase in water temperature in the summer leads to increased respiratory carbon loss which then slows plant growth. However such a suggestion is invalid for the following reasons:

i Mannitol and laminarin accumulate in Laminaria spp. during the summer (Black, 1948) whereas the hypothesis suggests utilisation of carbohydrate reserves.

ii Results from Luning (1971) and Johnston et al (1977) indicate maximum net photosynthesis during the summer; respiration is not then significantly higher than photosynthesis.

The absence of Laminaria from tropical waters and the slowing of growth of L. japonica once seawater temperatures exceed 20 °C (Tseng, Wu & Sun, 1957) provides some support for the adverse effects of high temperatures on Laminaria growth, but the mechanism has not yet been identified. Fortes & Luning (1980) reported a temperature optimum for growth of L. saccharina at 15 °C (over short-term - 1 week experiments). This, in conjunction with maximum seawater temperatures rarely exceeding 15 °C at the present study area on the east coast of Scotland (Drew, pers. comm.)

suggests that rising temperature is not involved in causing the spring growth rate decline and low summer growth rates of L. saccharina and L. digitata.

Neither photoperiod nor irradiance adequately account for the decline in growth rates after May; L. saccharina exhibits increased growth with increased daylength up to 24 hours light (Fortes & Luning, 1980) and summer underwater irradiances are not inhibitory to growth of L. longicruris (Anderson, Cardinal & Laroche, 1981).

The close relationship between the spring decline in phytoplankton biomass and the depletion of inorganic nutrients (particularly N and P) is well documented (Harvey, 1926; Ryther & Dunstan, 1971; Horwood, 1982). The effect of inorganic nutrients on seasonal growth of macroalgae is poorly understood. The timing of the nutrient seawater decline and the slowing of Laminaria growth has led to speculation that a causal relationship exists between the 2 factors (eg. Chapman & Lindley, 1980) but only in one instance has this been demonstrated directly. By in situ enrichment with sodium nitrate, Chapman & Craigie, (1977) showed that the spring growth rate decline of L. longicruris could be prevented and growth continued at high rates throughout the summer. However, the difference between L. longicruris and L. saccharina and L. digitata in the timing of, and the possible factors involved in the initiation of rapid growth suggests that responses of L. longicruris cannot be generally applied to the British Laminariaceae. Investigation of the role of nutrients in

the spring growth rate decline of L. saccharina and L. digitata is, therefore, necessary.

There is some evidence that summer seawater nutrient levels are limiting macroalgal growth. Indirectly this is indicated through a decline in tissue N content to a minimum level in mid-summer in Laminaria (Black, 1948; Haug & Jensen, 1954; Larsen & Jensen, 1957). Summer nutrient limitation of macroalgal growth has been shown directly through laboratory enrichment experiments on Alaria esculenta (Buggeln, 1974) and Fucus spiralis (Topinka & Robbins, 1976). These observations together with those of Chapman & Craigie (1977) confirm that nitrogen and also possibly phosphate (Buggeln, 1974) deficiency exists during the summer months and is likely to be a main cause of the low summer growth rates of Laminaria spp.

LOW AUTUMN AND EARLY WINTER GROWTH RATES

Arising from the premise of nutrient limitation of summer growth of Laminaria it might be expected that growth rates would increase in the autumn (September) as seawater nutrient concentrations increase as is seen in the autumn peak in phytoplankton biomass. This occurs in L. longicruris (Chapman & Craigie, 1977) but is not apparently found in L. saccharina or L. digitata, although there is some indication of a small autumnal growth rate increase in L. digitata (Cosson, 1967). Seawater temperatures are close to maximal and irradiance is high; the lack of Laminaria growth in September/October is unlikely to result from limitation of either of these 2 factors. Buggeln (1978)

suggested that reduced growth of Alaria esculenta in the late summer/autumn might be due to an endogenous rhythm in the yearly growth cycle. Assuming light, temperature, nitrate and phosphate (which are both increasing in the autumn in north temperate waters (Foster et al, 1978)) are not depressing growth, the possibility of an endogenous circannual rhythm or of senescence of the frond tissue (either endogenously or exogenously induced) is an important consideration requiring testing. It is proposed that irradiance and temperature are limiting growth in winter (indicated in results of Luning, 1971; Johnston et al, 1977; Fortes & Luning, 1980), hence the low growth rates during November and December; but this explanation alone seems inadequate since if these factors are limiting in November and December they must also be limiting in January and February. New growth would not then be expected to begin and increase during January and February. Lack of growth at the end of the year (November/December) provides further support for the occurrence of an endogenous circannual rhythm or for a loss of growth potential or senescence of the frond tissue.

Various areas of the control of the seasonal growth cycle of L. saccharina and L. digitata have only been sparsely investigated or assumptions made based on results from other species from very different locations. Particular aspects of the cycle requiring clarification are the importance of light in the control of new frond initiation and growth; the spring decline and low summer growth rates

including testing the hypothesis of nutrient limitation of Laminaria growth; later loss of growth potential and possible senescence of the frond tissue with season.

The major part of this work involved a rigorous test of the hypothesis that dissolved inorganic nutrients are of prime importance in determining frond growth rates of L. saccharina and L. digitata. The following experimental procedure was adopted: four sites which differed in their nutrient status (as predicted from domestic sewage input) were chosen for study and measurements were made at 4 periods in the annual growth cycle of Laminaria spp; during (i) the spring decline in growth rates, (ii) the low summer growth rates, (iii) low autumn growth rates and (iv) the development of the new frond in January.

The effects of nutrients on growth were investigated by estimating external and internal N and P levels and carbohydrate reserves throughout the year. Quantitative aspects, including kinetics, of nutrient uptake and growth during nutrient enrichment were studied during each of the 4 periods in the annual growth cycle mentioned above; testing the theories of nutrient limitation of spring and summer growth of Laminaria spp. and of loss of growth potential or senescence of the frond tissue during the latter half of the year.

Carbohydrate reserves were measured throughout the year to indicate availability for winter growth and to test the Harder:Knip hypothesis that carbohydrate reserves limit summer growth.

Growth responses to varied light and temperature were examined to investigate possible limitation of growth during November and December and to test the hypothesis of a photoperiodic control of new frond initiation. Estimation of the importance of the old frond to new frond growth in providing reserves and increasing the plant surface area involved removal of old frond reserves and reducing the surface area by excision, both in situ and in the laboratory and measuring rates of frond erosion in situ.

CHAPTER 1A

DISTRIBUTION AND ECOLOGY OF

L. digitata AND L. saccharina

Laminaria is one of the most important benthic genera in the sublittoral euphotic zone, inhabiting temperate and polar regions, mainly in the northern hemisphere.

Laminaria digitata (Huds.) Lamour extends from its southern limit of south Brittany (Gruet, 1975) northwards to Spitzbergen, Iceland, East Greenland and Nova Scotia (Gayral & Cosson, 1973). L. saccharina (L.) Lamour is common in the North Atlantic with a southern limit of south of Cape Cod (Sears & Wilce, 1975).

L. digitata is the main intertidal species of the Northeastern Atlantic but it is confined to the lowest 1-2 m of this zone, presumably because of its fast rate of water loss during emersion (Isaac, 1935). The lower limit is more variable depending on the geographical location. Where L. hyperborea is abundant it out-competes L. digitata because of the relatively stiff stipe of the former holding the lamina well above the substrate and reducing light penetration to these lower growing L. digitata. In these situations the lower limit of L. digitata growth will be pushed upwards. In some locations L. digitata has been reported to extend to below 20 m at Spitzbergen (Svendsen, 1959) and Nova Scotia (Edelstein, Craigie & McLachlan, 1969). L. digitata, presumably because the flexible stipe which reduces the leverage on the holdfast and makes it better adapted to wave action, grows well

in strong reversing currents which are less well tolerated by L. hyperborea (Ebling, Sleigh, Sloane & Kitching, 1960).

L. saccharina is the main competitor of L. hyperborea in the sublittoral zone. Walker (1947, 1950, 1954) concluded that the outcome of this competition was determined by the substratum, L. hyperborea requiring solid rock and L. saccharina being better adapted to unstable conditions. However, L. saccharina appears to be the successful species on solid rock when this is sufficiently sheltered either by depth or position (Kain, 1962, 1971; Jorde, 1966; Norton & Milburn, 1972; Tittley, Irvine & Jephson, 1976). The reason for this is not clear. L. saccharina grows in both exposed and sheltered locations on the west coast of the Atlantic (Lamb & Zimmermann, 1964; Mathieson & Fralick, 1972) but L. hyperborea presumably out-competes it on the northeast shores. L. hyperborea can grow in sheltered locations (Svendsen & Kain, 1971), although more slowly, but the rigid stipes would raise the fronds above the substrate and reduce light penetration to the shorter-lived, flexible-stiped L. saccharina, as presumably occurs with L. digitata. Therefore, the extent of L. saccharina even in sheltered locations is not clear. The adaptation of L. saccharina to unstable substrates is more understandable as the flexible stipe reduces leverage on any boulder to which it is attached, reducing the chances of it being turned over by water movement, while the flexibility of the stipe reduces the possibility of damage to the plant should the boulder be moved.

The lower limit of L. saccharina is similar to that of L. hyperborea where they occur together (Kain, 1962, 1971; Morton, 1968), extending down to a very variable depth, limited by substrate, grazing or irradiance. In clear water the lower limit may be at 34 m (Western Norway; Kain, 1971) or as little as 4 m in turbid water with poor light penetration (eg. Menai Strait; Knight-Jones, Jones & Lucas, 1957).

Although L. saccharina extends further into the sublittoral than L. digitata, only those L. saccharina sporophytes growing in the intertidal zone were used in field and laboratory experiments. In consequence, both L. digitata and L. saccharina are considered as lower intertidal species in the interpretation of results presented in this thesis.

CHAPTER 2

MATERIALS AND METHODS

2i SITES

Four sampling sites were chosen around the North-East Fife Coast (Fig. 2i) varying in the degree of exposure and nutrient enrichment.

The degree of exposure at each site was estimated using the technique of Grenager & Baardseth (1966); in which a transparent disc was placed with its centre on the sampling site and the number of "open" sectors (10° angle and radius of 7.5 km) are counted from that point. An open sector contains no land or shallows. The greater the amount of open sea, ie open sectors, the greater the degree of exposure.

The relative degree of nutrient enrichment was estimated by considering both domestic sewage (in terms of the population of the area to which the outlet serves) and the possible degree of land run-off.

Fifeness. Very exposed rocky shore subjected to strong wave action (Exposure index of 19). The rocks slope steeply into the sea. The seawater is generally turbid as sediment is kept in suspension because of the exposed nature of the site. There is probably a considerable amount of land run-off from the surrounding agricultural land, but only a small input of domestic sewage from the houses attached to the Coastguard Station. L. digitata is found in rockpools and at low water,

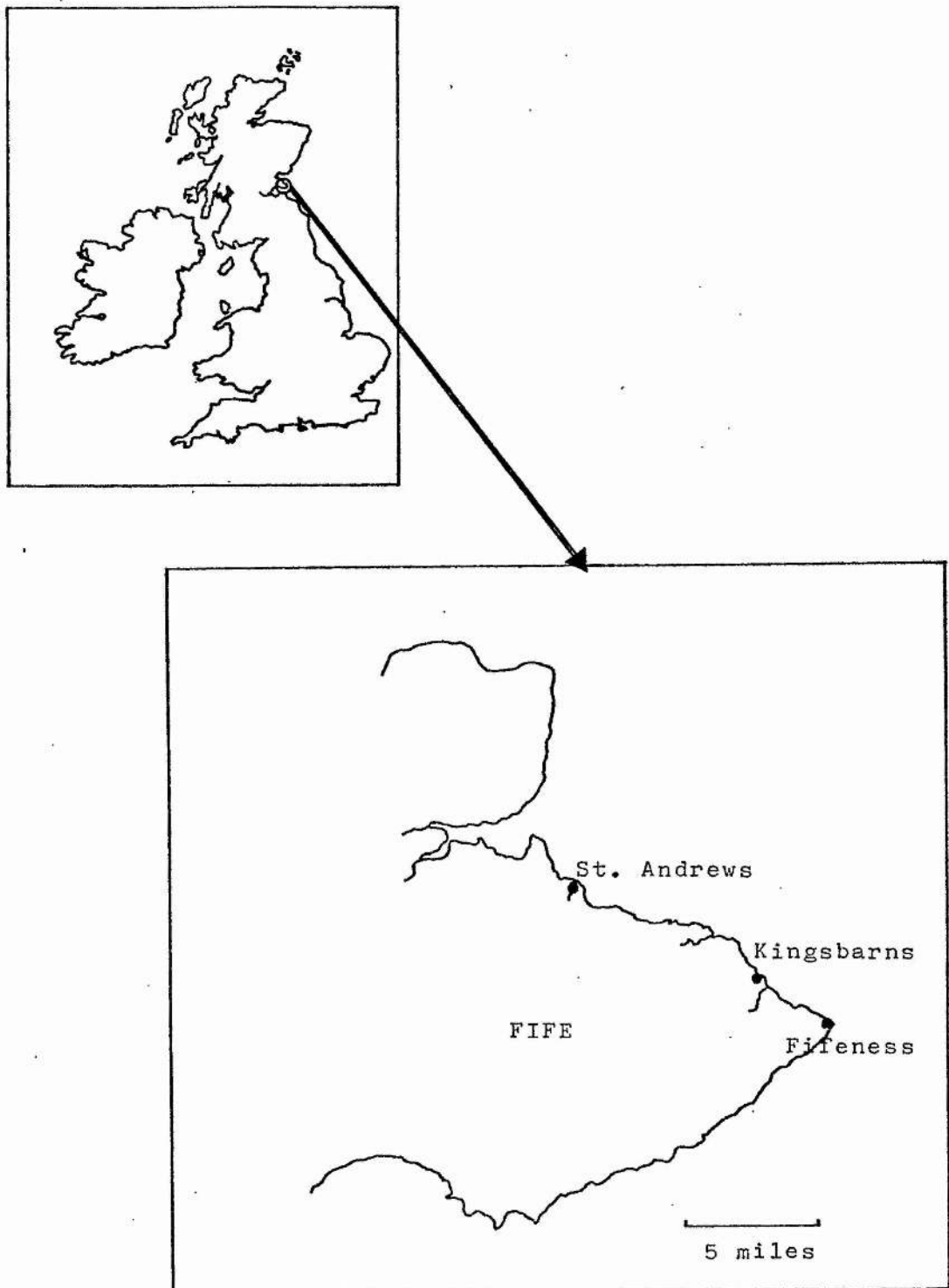


Fig. 2 i. Field sampling sites along the North-East Fife coast.

L. saccharina only in the more sheltered pools while Alaria esculenta dominates at low water.

Kingsbarns. Gently sloping shoreline with a coarse sandy substrate with long limestone reefs running perpendicular to the incoming waves. The site has an Exposure index of 13. Agricultural land probably results in considerable run-off and the domestic sewage outlet serves a population of approximately 200. L. digitata and L. saccharina are predominantly on the north-facing walls of the reefs.

St. Andrews. Gently sloping shoreline with the rocky substrate north of the pier consisting of reefs running parallel to the pier and perpendicular to the incoming waves, with rock, boulders or sand between the reefs. The site has an Exposure index of 12. The water is heavily polluted with domestic sewage (approximately 58,000 gallons/day) and as a result, is always turbid. Freshwater from the Kinness Burn, entering the harbour to the south of the pier is probably very significant after heavy rain. Laminaria predominates on the north-facing walls of the reefs and in pools.

St. Andrews Sewer. The sewage outlet pipe runs out from the north side of the pier, perpendicular to the rock ridges at St. Andrews, with the outlet situated at extreme low water of Spring tides (ELWS). The seawater is very turbid. This represents only a small sampling area with access limited to low spring tides only. L. saccharina and L. digitata are abundant in this

area.

To summarise: the sites can be placed in order of exposure and of nutrient enrichment. Fifeness is the most exposed site followed by Kingsbarns and St. Andrews as the least exposed, although there is little difference in exposure between the latter 2 sites. With regard to nutrient enrichment, the sewer site has the greatest enrichment, this is diluted slightly at the St. Andrews site, Kingsbarns is moderately enriched and Fifeness the least, although because of the greater degree of exposure and disturbance of the sediment at Fifeness, Kingsbarns and Fifeness may be similar in final nutrient levels despite the higher input of domestic sewage at Kingsbarns.

211 MEASUREMENTS IN THE FIELD

i SEASONAL GROWTH MEASUREMENTS

50 sporophytes each of L. saccharina and L. digitata were marked on the shore at Kingsbarns, Fifeness and St. Andrews, and due to limited access and limited time available for measurements, 20 of each species at St. Andrews Sewer site.

Plants were selected systematically for marking. Two of the perpendicular reefs were selected at St. Andrews and Kingsbarns and a small area of steep rock, with pools and an area of sloping beach at Fifeness. Groups of plants, usually 4-5 sporophytes were marked approx-

imately every 3 metres from low water up the shore along the reefs. At Fifeness, the plants were similarly marked in groups up the shore on the boulder beach and on the rock substrate, from the lowest pools up the shore.

Sporophytes which were heavily infested with Patina pellucida or Littorina littorea were not used, nor sporophytes where the frond was badly eroded or damaged by wave action.

The number of marked sporophytes was maintained by replacing those lost by marking another from the same group of sporophytes as that lost.

Sporophytes were marked by tying a 20 cm length of coloured, plastic coated wire around the haptera; individual plants were identified by a system of colour coding.

Linear growth rate of the frond in situ was measured by the punched hole technique of Parke (1948), using the apparatus described by Sundene (1964); a small brass plate with a 2 cm notch was fixed to the end of a centimetre scale and holes made in the scale at 2,4,6....20 cm. When marking the plants, the top of the stipe was placed in the notch and the scale pressed against the lamina. A hole, 2.5 mm in diameter was punched in the frond at 10 cm above the stipe/lamina boundary, using a sharpened length of steel tubing, pressed against a "formica" writing board. The distance of the hole from the transition zone of stipe and

lamina, was measured fortnightly in a similar way and a new hole punched at 10 cm.

L. digitata sporophytes were marked and measured from January 1979 to May 1981; L. saccharina from January 1980 to May 1981.

ii FROND EROSION EXPERIMENT

The rate of frond erosion at the distal end was measured using the method described by Chapman & Craigie (1978); 20 L. digitata and 20 L. saccharina sporophytes with fronds 50-55 cm in length were tagged at St. Andrews in October 1980. 10 holes, 5 cm apart were punched along the midline with the first hole at 5 cm from the transition of frond and stipe. The number of holes remaining in the frond at the beginning of each month until March 1981 was recorded to provide a measure of the rate of attrition of the frond tissue during the winter.

iii REMOVAL OF ACCUMULATED RESERVES BY EXCISION

The importance of reserves accumulated and stored during the summer and autumn in the mature, distal regions of the frond, to new frond growth the following year was investigated in L. digitata and L. saccharina. These reserves were removed in October 1980, by excising the frond 10 cm above the meristem of 20 L. digitata and 20 L. saccharina sporophytes growing at

St. Andrews. These plants were distributed among the groups already marked for seasonal growth measurements. Growth rate of fronds was measured monthly (until March 1981), as described above (i) and compared to growth rates of intact control sporophytes (intact fronds). The controls being those plants marked for seasonal growth measurements.

iv AGING OF MARKED PLANTS

In June 1981, all marked plants on the shore at the four sites were collected and aged. Cutting a disc of tissue (1 mm thick) from the stipe, just above the uppermost ring of haptera and counting the number of dark slow-growth rings present gave the minimum age (in years) of the sporophytes (method described by Kain, 1963).

2iii SELECTION OF PLANT MATERIAL FOR LABORATORY EXPERIMENTS AND ANALYSIS

Laminaria sporophytes selected for experimental manipulation and for analysis (of N, P and carbohydrate) must be representative of the plants marked for seasonal growth measurements. The heterogeneity of the shore prevents complete random collection of plants as being the most effective means. Instead sporophytes were chosen from areas where sporophytes were marked for seasonal growth measurements and specifically from

amongst the groups where several sporophytes had been marked. Very large (fronds > 70 cm length) and very small (fronds < 10 cm length) sporophytes and those heavily infested with P. pellucida and L. littorea, or severely eroded were avoided.

All L. saccharina and L. digitata for laboratory growth or uptake experiments were collected from Kingsbarns. Plant material for analysis of nitrogen, phosphate and carbohydrate was collected from all 4 sampling sites.

2iv EXPERIMENTAL PROCEDURE

i GROWTH EXPERIMENTS

Two methods of investigating growth of Laminaria were employed

- a) using discs of L. digitata and
- b) using whole sporophytes of
L. saccharina and L. digitata

All plant material used in growth and uptake experiments was collected from Kingsbarns.

(a) Discs 2.5 cm diameter were punched from the frond of L. digitata in the region of the meristoderm between the transition zone and the point at which the frond begins to digitate, or in the basal 10 cm, whichever was less. Discs were cut during mid-morning, cleaned of epiphytes by wiping with muslin and maintained in running seawater for 4 days at ambient daylength prior to the

to the experiment. The longitudinal and perpendicular axes of the discs were then measured to estimate disc area; only discs whose area lay within 1 standard deviation either side of the mean value were used in the growth experiments. The discs were then cut back to their original size and allowed to continue growth. Growth occurred all over and was not decreased by cutting back to the original size.

Samples of 15 discs were grown in 2 litre long-necked boiling flasks, the water circulated by a stream of compressed air supplied through glass tubing. The water in the flasks was changed every 2 days. Discs were measured for area (long and short axes) every 4 days and weighed every 2 days.

(b) Small sporophytes of L. digitata and L. saccharina (presumed age 1-2 years) were collected, the fronds were cut to 21 cm length and cleaned by wiping with muslin. The holdfast was cleaned by cutting off all but the uppermost hapteran whorl and careful wiping. Holes, 2.5 mm diameter were punched in the frond at 2,4,6...20 cm from the transition zone (using the apparatus described by Sundene, 1964).

The plants were grown upright in 50 cm lengths of perspex tubing (94 mm internal diameter) sealed at one end, with a capacity of 3 litres. The holdfast of the Laminaria was retained between the network of a circle of wide mesh plastic gauze slightly greater than the

tubing diameter placed at the base of the perspex tube. Water in the tubes was circulated by an air stream supplied to the bottom of the tube by a fine drawn-out glass pipe; the air stream caused the plant to maintain an upright flattened position with maximum surface area for light absorption.

The positions of the holes and the fresh weights of the plants were measured every 2 days and the water changed at the same time, for the duration of the experiment (14 or 20 days).

GROWTH CHAMBERS

All growth and uptake experiments were carried out in growth chambers illuminated by 4 cool white fluorescent tubes 55 cm above the bench surface ($99 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$). The flasks or tubes stood in water baths cooled by a Churchill Thermo-Chiller Circulator; in order to maintain ambient seawater temperature in the flasks or tubes the circulating water temperature was set at 6°C below that temperature required. The water baths were surrounded by foil-covered reflecting screens and the growth chamber was enclosed in black polythene. Photoperiod was maintained as ambient unless otherwise stated. A small compressor provided an air supply to each flask or tube.

CULTURE MEDIUM

Seawater sterilised by boiling was used in the growth experiments and the natural levels of nitrogen and phosphate could be supplemented by the addition of phosphate and/or nitrate from stock solutions to give a mean winter concentration of $15 \mu\text{g-at. NO}_3\text{-N/l}$ and $3.0 \mu\text{g-at PO}_4\text{-P/l}$. Nitrate was added as the nitrogen source; but nitrate, nitrite, ammonium and urea were also added separately for investigations of growth on different nitrogen sources.

Water with very low levels of nitrogen and phosphate was obtained by pretreatment with L. digitata. Two fibre-glass tanks (1.05 m x 0.60 m x 0.27 m deep) were filled with seawater from the laboratory circulatory system (capacity of each tank 171.1 l). The tanks were situated outside in a shady courtyard and covered with polythene sheeting on a wooden frame. The temperature of the water, although static, never exceeded 17°C . Six L. digitata sporophytes per tank, with frond length approximately 50 cm were cleaned of epiphytes by wiping, senescent tissue removed and the holdfast cleaned. The plants were attached to stones using elastic bands; within 1-2 months the Laminaria had produced new haptera and attached directly to the stones. Plants were replaced as necessary.

The algae depleted the water of N and P within 2 days (during the summer) and 4 days (at winter N and

P concentrations). This depleted seawater was sterilised by boiling and used in growth and uptake experiments, being supplemented by the trace element solution of Provasoli, McLaughlin and Droop (1957) at half strength.

2iv EXPERIMENTAL PROCEDURE

ii NUTRIENT UPTAKE EXPERIMENTS

Time course experiments over 24 hours were carried out to investigate the uptake of nitrate, nitrite, ammonium and phosphate by L. digitata and L. saccharina. Experiments using L. digitata used tissue discs cut from the meristem; 15, 2.5 cm diameter discs were incubated per 2 l flask whilst experiments with L. saccharina used a single small sporophyte per 3 l perspex tube. In all experiments there were 3 replicates/treatment.

Uptake of nitrogen and phosphate was measured as loss from the seawater. Three flasks or tubes were removed every 3 hours during the course of the experiment, the alga or discs removed and the seawater analysed for remaining N and P.

2iv EXPERIMENTAL PROCEDURE

iii PHOTOSYNTHETIC MEASUREMENTS

Photosynthetic rates of L. digitata and L. saccharina were measured using the Oxygen Method (Winkler) described by Drew & Robertson (1974) and Robertson (1976).

Five discs (1 cm diameter) or a single strip of tissue, approximately 50 mm x 3 mm wide (actual area determined by tracing the outline on to graph paper and counting the squares) were incubated in McCartney Bottles (28 ml screw-capped universal containers). Two sets of tissue were cut from the same area of the frond, one set (ie 5 discs or one strip) was incubated in the light, the other, in the dark (by being covered in foil) to allow calculation of Net Photosynthetic production after respiration has been accounted for.

Bicarbonate was added to seawater, sterilised and with most of the oxygen removed by boiling, to give a final concentration of 3 moles.m^{-3} . The McCartney bottles were filled and sealed underwater containing the algal tissue and several glass beads to ensure mixing of the contents of the bottle during incubation.

Five replicates/treatment were incubated and analysed.

The pairs of bottles were incubated horizontally for 2 hours at ambient seawater temperature or at 15°C (for maximum photosynthetic rates or photosynthetic capacity measurements) in an agitating water bath illuminated by 2 quartz halogen lamps (40 cm above the water bath) at $560 \text{ }\mu\text{E.m}^{-2}.\text{sec}^{-1}$.

The water in the McCartney bottles was then analysed for dissolved oxygen using the Winkler method modified by Drew & Robertson (1974) and the chemical reactions of Strickland & Parsons (1968). See Appendix 1.

2iv EXPERIMENTAL PROCEDURE

iv SCANNING ELECTRON MICROSCOPY

To investigate the best method for removal of epiphytes from the surface of Laminaria prior to growth and uptake experiments, 3 treatments were examined:

- a) Untreated tissue
- b) Tissue cleaned by wiping with muslin
- c) Tissue cleaned by running under 10 l of filtered seawater

The tissue (from frond, stipe and holdfast) of L. saccharina and L. digitata was cut into small pieces (2 mm lengths) and prepared using the method of Howard-Williams, Davies and Cross (1978) with several modifications. The tissue strips were placed in 5% glutaraldehyde in seawater, dehydrated through the alcohol series (30,50,70,90,100,100%) followed by 2 changes of 100% amly alcohol. The tissue was critical point dried, mounted on specimen stubs and sputter coated with gold (Edwards High Vacuum Ltd Coating Unit. Model 12E6/1688) and viewed on a Cambridge Stereoscan 600 Electron Microscope. Representative areas were selected and photographed.

The surface of the untreated tissue was seen to be densely covered in bacteria with the occasional diatom. Cleaning in running seawater was ineffective but wiping with muslin removed almost all the epiphytes from the frond, stipe and holdfast surface of both L. digitata

and L. saccharina. The results are discussed further in chapter 4.

2v ANALYTICAL PROCEDURES

i ENVIRONMENTAL ANALYSES

a) Water Analyses

Water samples were collected on the incoming tide just after low water of Spring tides in 5 x 400 ml polythene bottles. The water was analysed for nitrite, nitrate, ammonium and phosphate within 2 hours of collection or samples were frozen, analyses were then carried out after thawing to room temperature.

Nitrite. The nitrite in 50 ml seawater sample was assayed using the method of Strickland and Parsons (1968); the nitrite reacts with sulphanilamide in an acid solution producing a highly coloured azo dye on reaction with N(1-naphthyl)-ethylene diamine dihydrochloride solution. The optical density of the dye was measured in 10 cm glass cuvettes on a Corning Absorptiometer (Filter 604) against distilled water. Distilled water blanks and standard nitrite solutions were analysed in a similar way. (Details in Appendix 2)

Nitrate. Using the method of Strickland and Parsons (1968) the nitrate in a 90 ml seawater sample is reduced to nitrite over amalgamated cadmium filings. The nitrite is then assayed as described above. The optical density of the azo dye was measured in 1 cm glass cuvettes

on a Pye Unicam SP6-450 UV/VIS Spectrophotometer at 543 nm. (Details in Appendix 3)

Ammonia. The ammonia in the seawater sample is oxidised to nitrite by the addition of sodium hypochlorite solution. The reaction is allowed to proceed for 4 hours at room temperature before the excess oxidant is destroyed by addition of sodium arsenate solution (Strickland & Parsons 1968). The nitrite is assayed as above, the optical density being measured in 1 cm glass cuvettes at 543 nm. (Details in Appendix 4)

Phosphate. Reactive low levels of phosphate were measured using a modification of the method described by Strickland & Parsons (1968) and Hellebust & Craigie (1978). To a 50 ml seawater sample in a conical flask, 5 ml of mixed reagent (Ammonium molybdate solution; sulphuric acid solution; ascorbic acid solution and potassium antimonyl tartrate solution (as in Strickland & Parsons, 1968)) is added and the solution shaken. After 15 mins at room temperature the optical density of the blue phosphomolybdate compound is measured in 10 cm glass cuvettes on a Corning Absorptiometer (red filter 609). Distilled water blanks and standard phosphate solutions were analysed in a similar way. (Details in Appendix 5).

b) Surface Seawater Temperature

Surface seawater temperature was measured fortnightly at St. Andrews on the incoming tide just after low water of spring tides.

c) Light Measurements

Total weekly irradiance was measured using an integrating dome solarimeter light detector attached to the highest point on the laboratory roof, connected to a digital counter operated by a 12 V battery. The counter was reset after dark every Thursday. Readings were converted to PhAR assuming PhAR to be 45% of the total short wave radiation (Drummond, 1960; Jupp & Spence, 1977).

2v ANALYTICAL PROCEDURES

ii PLANT ANALYSES

a) Carbohydrates

Mannitol and laminarin were extracted monthly during 1980 from L. digitata and L. saccharina from the 4 sampling sites. Alginic acid was extracted and analysed from January-March 1980. In all the plant analyses there were 3 replicates/treatment.

i MANNITOL

Two discs, 2.5 cm diameter, were cut from the frond with centres at 2 cm and 20 cm from the junction of stipe and frond; each disc was treated separately. The disc was cut into small pieces, immersed in sufficient cold 80% ethanol to cover it in a 25 ml plastic screw-top vial. Extraction of the soluble compounds was completed with 3 additions of hot 80% ETOH followed by one wash in cold ETOH. The washings were added to the

total extract and made up to 25 ml with 80% ETOH. The extracted plant material was dried at 80 °C for 24 hours to determine extracted dry weight. A 5 ml aliquot of the alcohol extract was removed to a tared 25 ml glass vial, the extract was evaporated to dryness on a rotary evaporator at 75 °C. The vial was then weighed to give the dry weight of the alcohol soluble substances. Addition of the 2 values gave the total dry weight of the plant material discounting the loss of any volatile substances (Holligan & Drew, 1971). (Details in Appendix 6i)

ii LAMINARIN

The laminarin is hydrolysed to glucose and analysed by Gas-Liquid Chromatography. The extracted dry tissue from the mannitol analysis was hydrolysed with 2.5 ml NH_2SO_4 for 6 hours in foil-capped test-tubes in a boiling water bath. The hydrolysate was decanted, the algal tissue was washed with distilled water, the hydrolysate and washings then made up to 10 ml with mixing. 2 ml of the hydrolysate solution were neutralised with barium carbonate powder (neutrality determined using pH paper or the point at which no further carbon dioxide is liberated). The solution was centrifuged for 10 minutes (setting 4, MSE bench centrifuge), 1 ml of the supernatant was removed and evaporated to dryness on a rotary evaporator with the water bath at 80 °C. (Details in Appendix 6ii)

Quantitative analysis of the mannitol and glucose was carried out using Gas Liquid Chromatography (GLC). A Pye Unicam, series 105 (model 15) Analytical Gas Chromatograph with a flame ionization detector and a single glass column (5' by 0.25" internal diameter) was used, in conjunction with a Phillips (PM 8220) pen recorder.

Column Packing and support material. The solid support phase was acid washed, silicanised Diatomite 'C' (mesh 60-70) and the liquid stationary phase, 2% methyl phenyl silicone gum (SE 52), prepared as described by Holligan & Drew (1971). The column was packed by applying a moderate vacuum to the outlet.

Preparation of volatile derivatives. The volatile trimethyl-silyl (TMS) derivatives were prepared by redissolving the dried ethanol or acid extracts in 0.85 ml pyridine and adding, within 5 minutes, 0.1 ml hexamethyl-disilazane (HMDS) and 0.05 ml trimethylchlorosilane (TMS) to give a reaction mixture volume of 1.0 ml. (Sweeley et al, 1963) After vigorous shaking and warming the reaction mixture was allowed to stand for at least 3 hours at room temperature before analysis.

Injection of samples and column programme. 10 μ l samples of silylated derivatives were injected rapidly into the column using a Scientific Glass Engineering Syringe with 11.75 cm stainless steel needle, at 140 °C;

using a temperature programme 140-250 °C in 8 °C/min increments.

Carrier gas was Oxygen free nitrogen (70 psi at the cylinder head) with 5 psi at the column and a flow rate of 60 ml/min. Hydrogen (12 psi; 30 ml/min) and air (60 psi; 500 ml/min) were supplied to the flame ionization detector.

Identification and quantitative estimation of peaks.

The retention time from peak apex to the front of the solvent peak was measured and similar retention times to authentic standards were considered as evidence of chemical identity. Peak height was linearly related to mannitol concentration above 0.5 mg/ml, thus mannitol content of the algal tissue was estimated using measurements of peak height only. With laminarin, the concentration of α and β glucose was estimated from measurements of peak area, determined as Peak height x width of the peak at $\frac{1}{2}$ peak height (Ball et al, 1967).

iii ALGINIC ACID

Frond tissue (transverse strip cut at 0-5 cm and 15-20 cm above the transition zone) was dried on foil at 80 °C for 24 hours followed by grinding. Alginic acid was extracted according to the method of Cameron, Ross and Percival (1948) in which the dried, ground tissue is treated with dilute sulphuric acid followed by sodium carbonate. The sodium alginate thus formed, is converted to calcium alginate by addition of calcium

chloride. This is followed by acidification to remove the calcium ions, followed by treatment with calcium acetate, the alginate and calcium acetate react, releasing acetic acid. The amount of alginic acid is estimated by determining the quantity of acetic acid liberated from the calcium acetate solution using phenolphthalein as the indicator. (Details in Appendix 6iii)

b) Tissue Nitrogen Analysis

Internal inorganic and organic nitrogen was analysed monthly during 1980 from L. digitata and L. saccharina from the 4 sampling sites.

Two discs, 2.5 cm diameter, were punched from the frond with centres at 2 cm and 20 cm above the transition zone. Each disc was treated separately, with 3 replicates/treatment.

Inorganic nitrate was extracted by the method of Chapman & Craigie (1977), reactive nitrate was then determined according to Strickland & Parsons (1968), (see nitrate water analysis above).

Organic nitrogen was determined in the ethanol extract and in the extracted dried tissue after conversion to ammonia by Kjeldahl digestion (Strickland & Parsons, 1968). (Details in Appendix 7)

c) Tissue Phosphate Analysis

Tissue phosphate content of L. saccharina and

L. digitata was analysed monthly during 1980 from the 4 sampling sites.

Two discs, 2.5 cm diameter, were punched from the frond with centres at 2 cm and 20 cm above the transition zone. Each disc was then treated separately, analysis was completed with 3 replicates/treatment.

The disc was oven dried, ground followed by hydrolysis in 2.5 ml NH_2SO_4 in a boiling water bath for 4 hours. 0.1 ml of the hydrolysate was diluted to 200 ml with distilled water and analysed for reactive phosphate (see phosphate water analysis above). (Details in Appendix 8)

CHAPTER 3THE INFLUENCE OF TEMPERATURE AND LIGHT
ON SEASONAL GROWTH OF LAMINARIA

INTRODUCTION

The marked seasonal variation in linear growth rate of the frond in species of Laminaria has been known since the early observations of Setchell(1900)(see Kain, 1979) but it was not until the punched hole technique was introduced by Parke(1948) that increase in frond length could be measured accurately. Her method demonstrated a clear variation in seasonal growth of L. saccharina; spring growth begins in January, increases rapidly to a maximum in May and declines after this. The year could then be divided into a "period of rapid growth" (January to June) and one of "slow growth" (July onwards).

Growth rates of the alga cannot be looked at in isolation since the observed rate results from the interaction of the endogenous controls (senescence, internal reserves of carbohydrates, N and P) and the exogenous controls (light both in terms of irradiance and photoperiod; temperature and nutrients). Nutrients (particularly N and P) and the internal reserves are investigated in relation to seasonal growth in Chapters 4-7: the effects of temperature and light are examined below.

The apparent paradox which these large brown algae have of maximum linear expansion and growth in spring when seawater temperature, photoperiod and irradiance are all

lower than in the summer has usually been interpreted by the Harder:Knierp hypothesis (Knierp, 1914; Harder, 1915; Gessner, 1955). This suggests that the increase in water temperature in the summer leads to increased respiratory carbon loss which then slows plant growth. More recently, however, it has been concluded that maximum net photosynthesis occurs in the summer in these algae (Luning, 1971; Johnston et al, 1977) and this hypothesis no longer provides an adequate explanation for the decline in Laminaria growth rates during the summer.

The slow rates of growth during November/December may result from low (and possibly limiting) seawater temperatures but increasing growth in January cannot readily be interpreted as a response to seawater temperature. Temperature is decreasing to a minimum in February/March and is, therefore, more likely to be limiting growth at this time than providing the "trigger" for rapid spring growth. Little experimental work has been carried out on the relationship between temperature and growth rate in macroalgae (see review: Gessner, 1970) probably because of the difficulties in handling these larger algae. Fortes & Luning (1980) attempted to fill this gap by undertaking an extensive study of the growth/temperature relationships of 21 species of macroalgae (including L. saccharina) in the North Sea and adjacent waters. Their results were interpreted in relation to the geographical distribution of the algae and not to the possible effect

of seawater temperature in influencing seasonal growth rates. For this reason, the temperature/growth relationship of L. saccharina is investigated in this context.

In a similar manner to temperature, the decline in growth rates during the summer (May onwards) cannot readily be seen as a direct response to increasing light (photoperiod and irradiance). Growth of L. saccharina has been shown to increase with increasing photoperiod up to continuous light (experiments were carried out during the summer: Fortes & Luning, 1980), however, growth rates increased with increasing irradiance to $110 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ but the highest irradiance used in the experiment ($250 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, equivalent to $50 \text{ W} \cdot \text{m}^{-2}$) was clearly inhibitory to growth. This irradiance is very low compared to normal summer irradiances and may suggest that light is limiting to growth during the summer. High light was inhibitory to photosynthesis in the subtidal L. hyperborea (where photosynthetic rates were reduced to about 50% when the alga was brought near the sea surface for 5 hours at $250\text{--}300 \text{ W} \cdot \text{m}^{-2}$) but no such inhibition was found for L. digitata (Drew, 1974). The effect of light on growth is therefore, not clear; growth may be inhibited by high light (irradiance) during the summer and low light (both photoperiod and irradiance) may limit growth later in the year (November/December).

Spring growth begins in January at a time when irradiance is low and often inadequate for photosynthetic

production to support growth rates (Luning, 1971; Johnston et al, 1977). The factor(s) which trigger the initiation of spring growth in Laminaria have never been adequately established. In L. longicruris, ambient nitrate concentration appears to be important since fast growth starts soon after ambient nitrate levels rise dramatically and growth slows sometime after it has fallen again (there is a lag period of about one month: Chapman & Craigie, 1977). In L. digitata and L. saccharina the pattern is less clear-cut: growth here begins in January 3 months after the seawater nitrate concentration increases in the autumn. It is suggested that light (photoperiod and irradiance) which is increasing after the shortest day, may act as the "trigger" for the initiation of rapid spring growth and this was investigated as described below by growth/photoperiod experiments on L. digitata and L. saccharina in December.

The new flush of meristematic growth, once started is maintained and increases, despite possible light and temperature limitations on production and growth. The alga must presumably be utilising carbohydrate reserves already present in the frond tissue. Several questions arise from this supposition:

- i) Are carbohydrate reserves adequate to support growth?
- ii) Is a marked decrease in carbohydrate evident?
- iii) Are carbohydrate reserves accumulated

in the frond during the previous summer actually available for spring growth? (ie. What proportion of the summer tissue is lost by distal erosion?)

Chapman & Craigie (1978) concluded that carbohydrate reserves formed during the summer did not play a significant role in the maintenance of growth of L. longicruris in January/February. However, in this species there appears to be sufficient photosynthetic carbon fixation to support growth demands throughout the year, except during November (Hatcher et al, 1977); as a result, stored carbohydrate reserves are probably only utilised as a carbon source during this one month. In addition, the high growth rates of L. longicruris in October/November (over 50% of the maximum April/May rates) probably utilises reserves rapidly and distal attrition would remove carbohydrate-rich tissue very rapidly. It is not clear in L. saccharina and L. digitata when or for how long light is below the compensation point for photosynthesis, although it is considered to be inadequate to support frond growth in December/January (Luning, 1971; Johnston et al, 1977). There may then be a greater demand on reserves by these species than for L. longicruris, and the slower growth rates in November/December in L. digitata and L. saccharina possibly suggest that a greater proportion of the distal tissue will remain intact by January (assuming rate of attrition to be simply proportional

to frond area). The importance of carbohydrate reserves in spring growth of L. hyperborea has been demonstrated by Luning (1969) and later evidence (Schmitz et al, 1972) indicated relatively rapid translocation of reserves (mainly mannitol) from the old tissues to the actively growing meristem. Since the pattern of reserve carbohydrates is similar in L. saccharina and L. digitata as in L. hyperborea it is probable that reserves are also important in supporting spring growth in the former 2 species. Whether carbohydrate reserves are sufficient to support growth at this time is discussed in Chapter 7, while data is presented below on the proportion of summer produced carbohydrates which remain available to the alga in January. If the alga is utilising reserve carbohydrates, then the old frond is presumably retained for this purpose and normal spring growth rates would not occur in its absence. The old frond may also have a number of additional roles including increasing the area for nutrient uptake, increasing the photosynthetic area of the alga and providing reserves of N and P in addition to carbohydrates.

The following results aim to investigate the temperature/growth and light(photoperiod)/growth relationships of L. saccharina and L. digitata during the year, and photoperiod is suggested as the possible "trigger" for the initiation of spring growth in January. The role of the old frond in spring growth is examined by experimental manipulation of reserves by excision and measurements

Fig. 3 i.

Mean Linear
growth rate of
the frond of
L. saccharina
during 1980-1981.

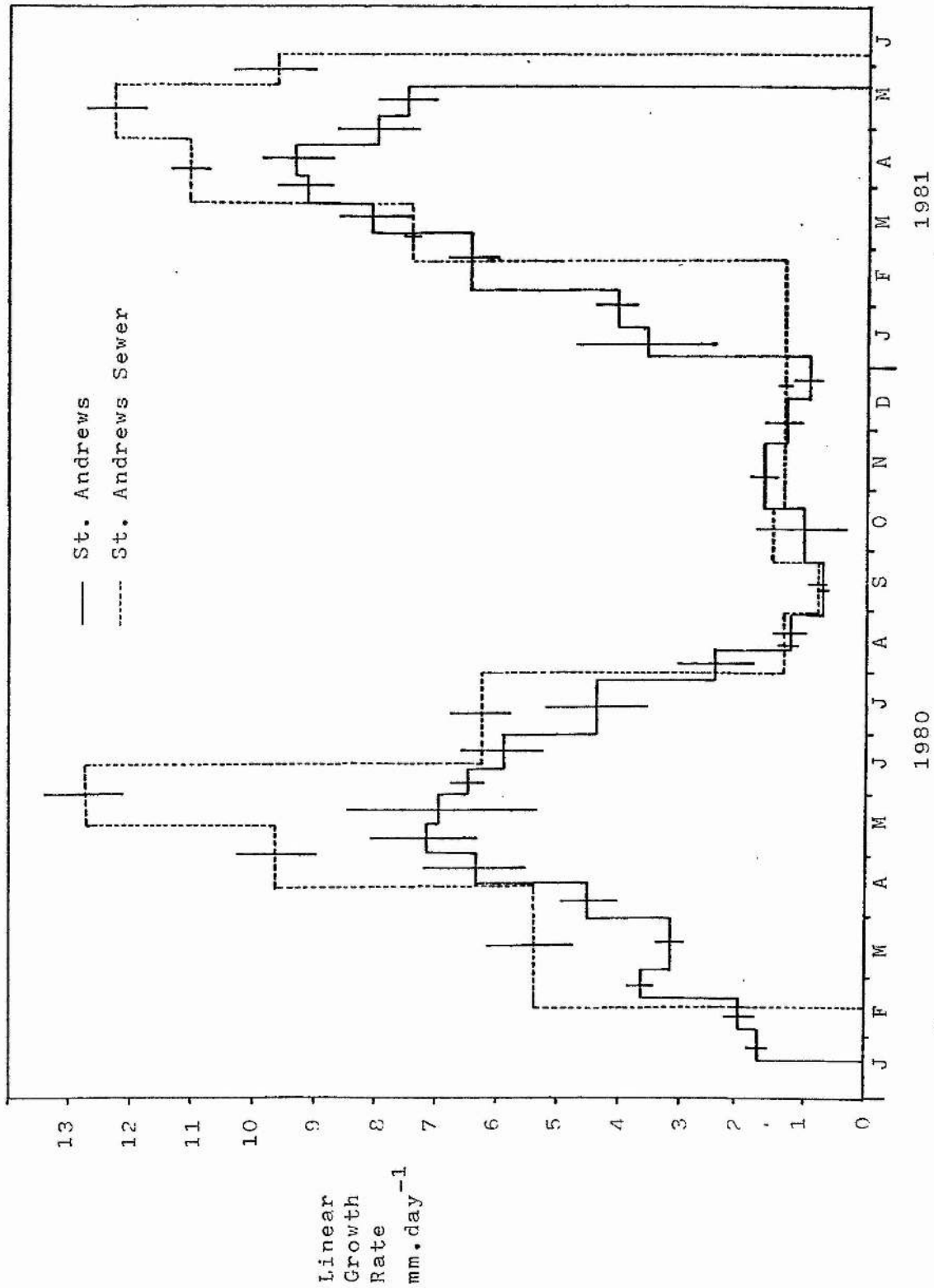


Fig. 3 ii.

Mean Linear growth
rate of the frond
of L. saccharina
during 1980-1981
at Fifeness and
Kingsbarns.

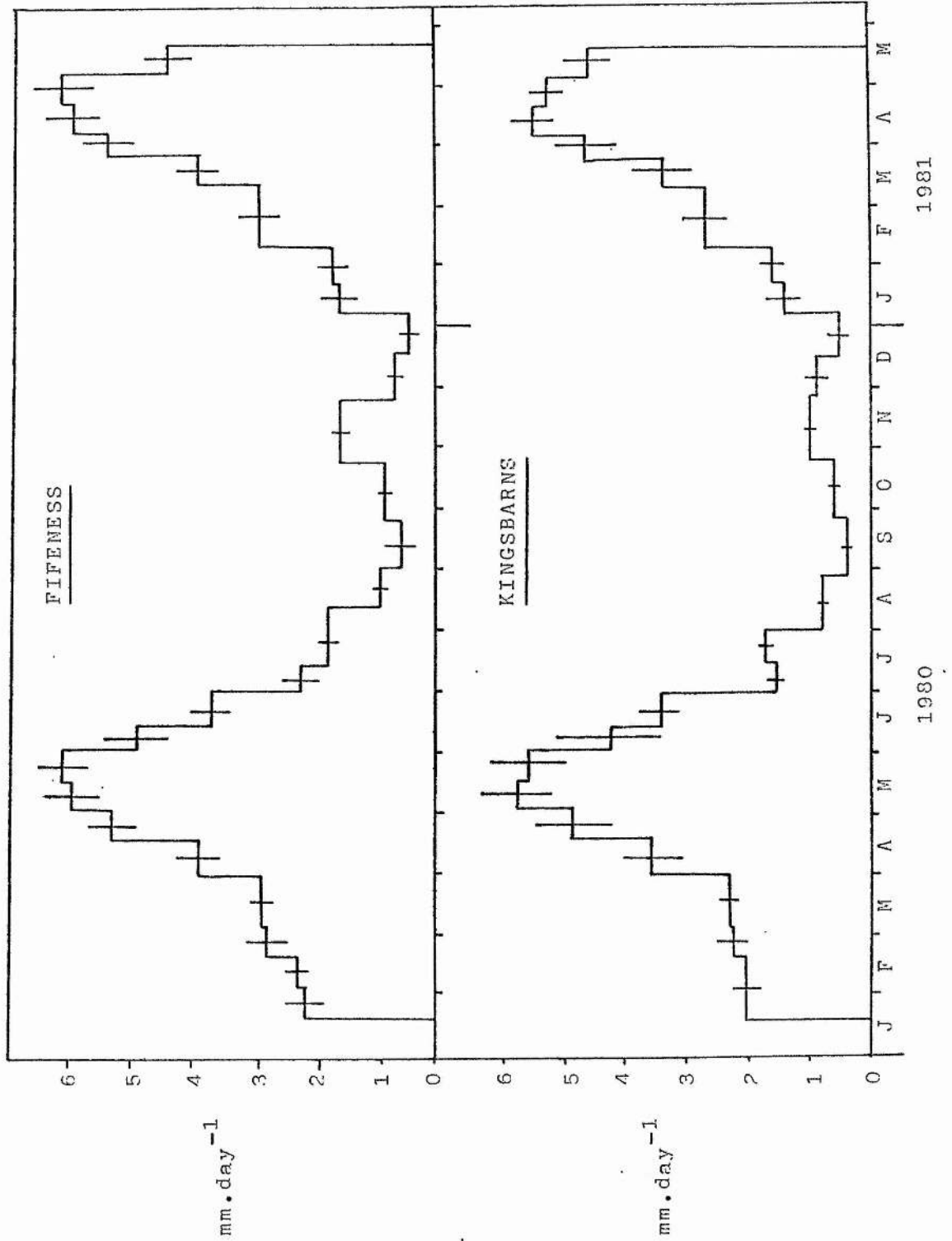


Fig. 3 iii.

Mean Linear Growth
Rate of the frond of
L. digitata at
St. Andrews and
St. Andrews Sewer
during 1979-1981.

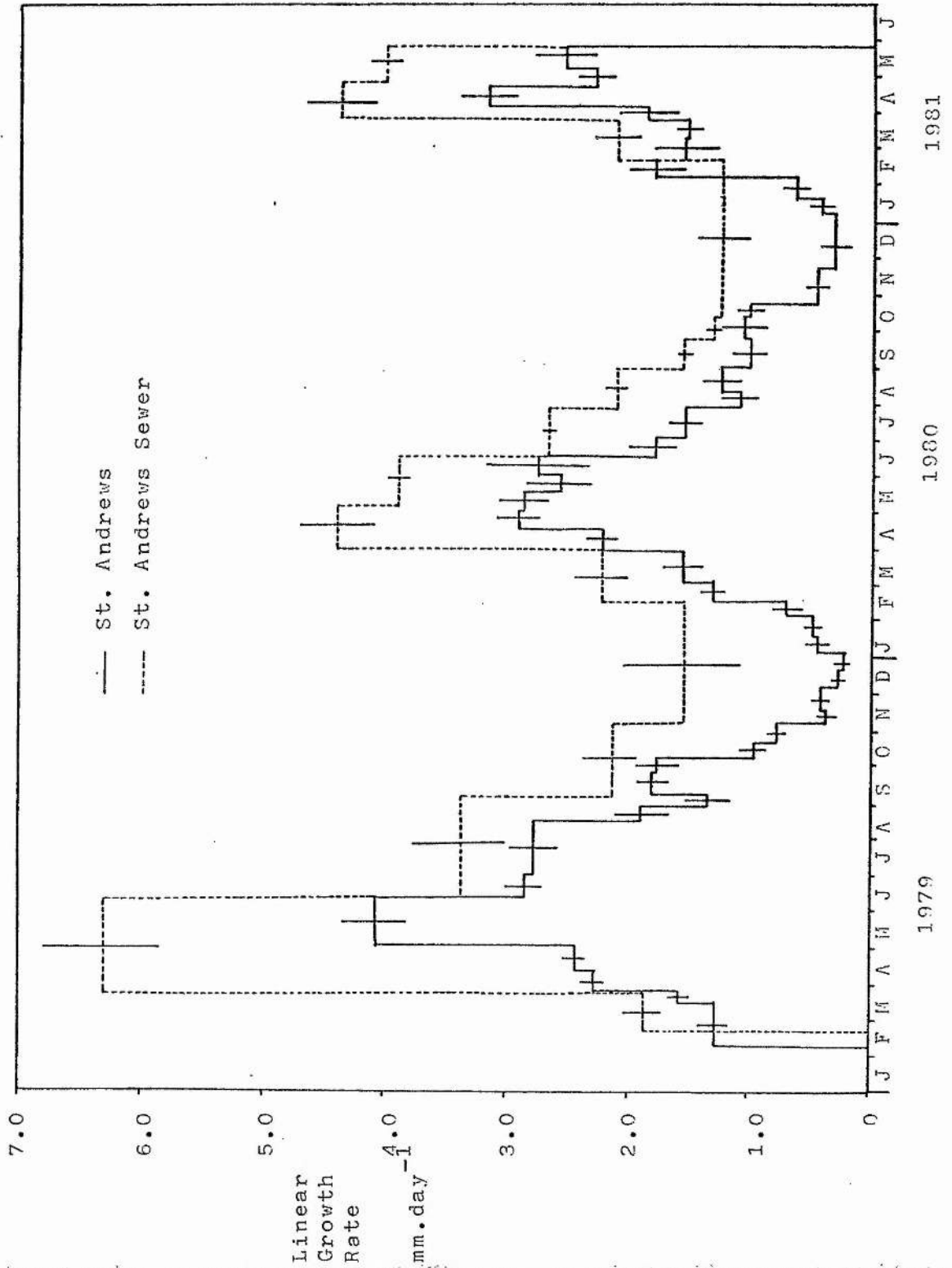
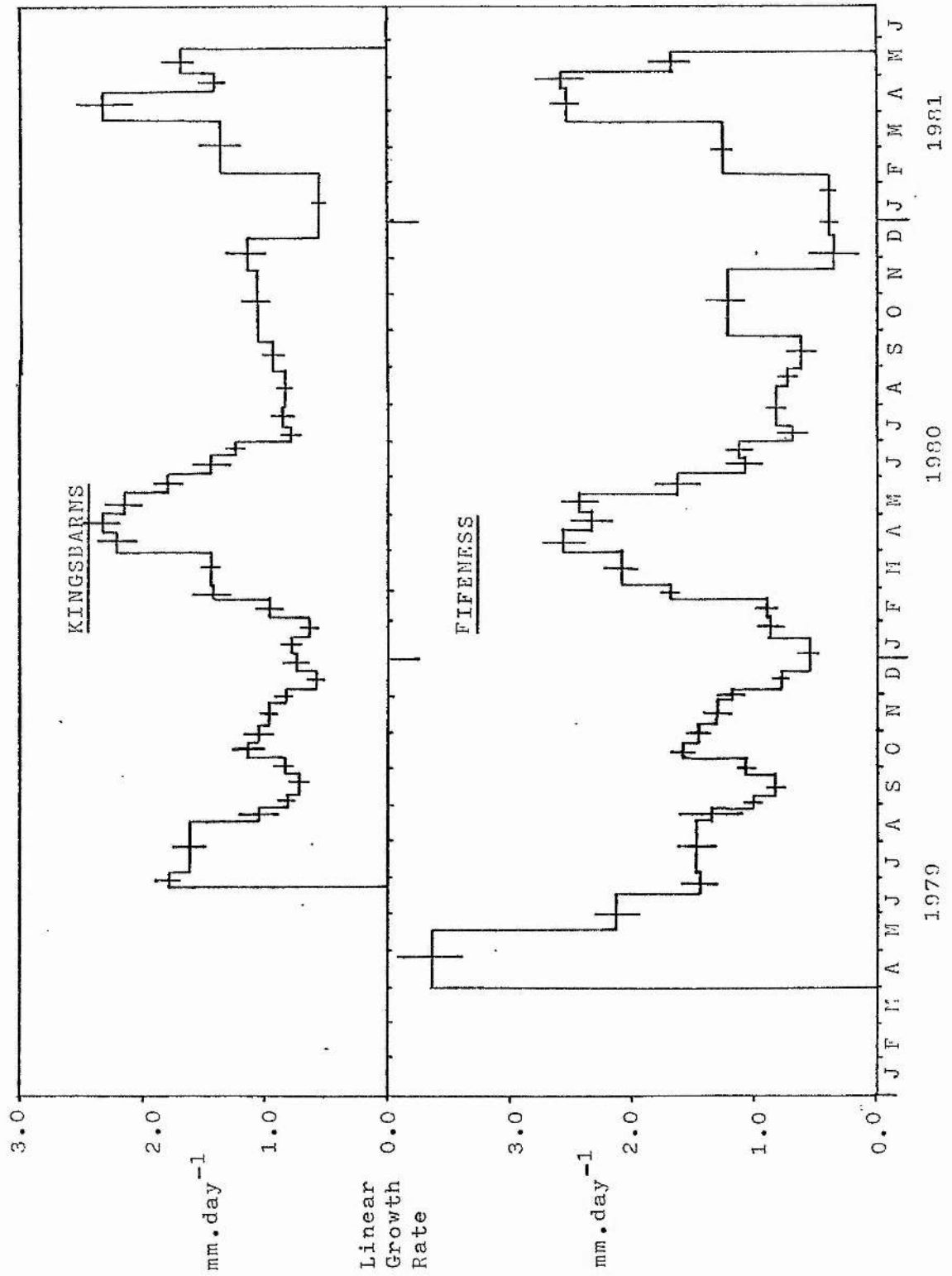


Fig. 3 iv.
Mean Linear Growth
Rate of the frond of
L. digitata at
Kingsbarns and
Fifeness during
1979-1981.



are made of the proportion of summer accumulated carbohydrate-rich tissue which is available for spring growth. The additional roles of the old frond (increasing surface area for nutrient uptake and photosynthesis and providing reserves of N and P) are also studied.

RESULTS

1 SEASONAL GROWTH

a Seasonal growth of *L. saccharina* and *L. digitata*

Both *L. saccharina* (Figs 3 i-ii) and *L. digitata* (Figs 3 iii-iv) show the same pattern of growth over the year. Linear growth rates of the frond begin to increase during the second week in January, rising rapidly to a peak in late April/May then falling to low rates during the summer and autumn. The year can, therefore, be divided into the 2 growth periods described by Parke (1948); a "period of rapid growth" from January to June, and one of "slow growth", from July onwards. Both species show a slight increase during September/October but growth rates then decline to a minimum in December before the following year's growth begin. This autumn shoulder represents a significant increase in growth rates in *L. saccharina* ($P < 0.05$) but not so in *L. digitata*. This shoulder is very much more pronounced in *L. digitata* at Fifeness and Kingsbarns (Fig. 3 iv).

L. saccharina grows at more than twice the rate of *L. digitata* (the spring maximum in 1980 is 2.94 mm.day^{-1} by *L. digitata* and *L. saccharina* respectively 7.15 mm.day^{-1}).

at St. Andrews); this may be partly attributed to the method of measurement, since an area of tissue produced as a narrow band (as in L. saccharina) will register a faster linear rate of growth than if the same amount became a broad band (as in L. digitata).

b The Effect of Age on Frond Growth Rates

After a peak in the spring, frond growth rates show an overall decline throughout the year. This decline may represent a senescence effect as the annually-produced frond ages, but there may also be an effect of plant age shown by a declining growth potential or declining maximum linear growth rate achieved by fronds produced in successive years by the same plant. Using plants which have been marked for 2-3 years it is possible to compare the maximum growth rates achieved by the same plant in successive years. Few L. digitata plants have remained for the whole 3 year period and growth measurements at Kingsbarns and Fifeness did not begin until after the spring maximum in 1979, but Table 3 i, shows the maximum growth rates achieved during 1980 and 1981 by L. digitata of the minimum age indicated at the time of collection in 1981.

With few exceptions, growth rates in 1981 were lower than in 1980, but it is difficult to assess whether this is simply an effect of age or caused by changes in environmental conditions. During 1980, with the exception of the 10 year old plant, maximum frond growth rates decline with increasing age, but results are very much more variable

Table 3 i. The effect of age on frond growth rates of L. digitata. Maximum linear growth rates (mm.day^{-1} over 2 weeks in May) achieved by the same plant (of the minimum age in 1981) in successive years (1980 + 1981). (Mean \pm SE)

Minimum Age (yrs)	No. of Plants	1980 mm.day^{-1}	1981 mm.day^{-1}
3	4	3.74 ± 0.81	2.95 ± 0.64
4	3	3.24 ± 0.45	3.32 ± 0.12
5	8	3.00 ± 0.36	1.83 ± 0.20
6	4	2.84 ± 0.47	2.21 ± 0.36
7	-	-	-
8	1	2.46	2.61
9	1	2.35	1.57
10	1	3.57	3.29

during 1981. However, generalisations are difficult with such a small sample size (22 plants in total with only one plant in each of the 8, 9 and 10 age groups).

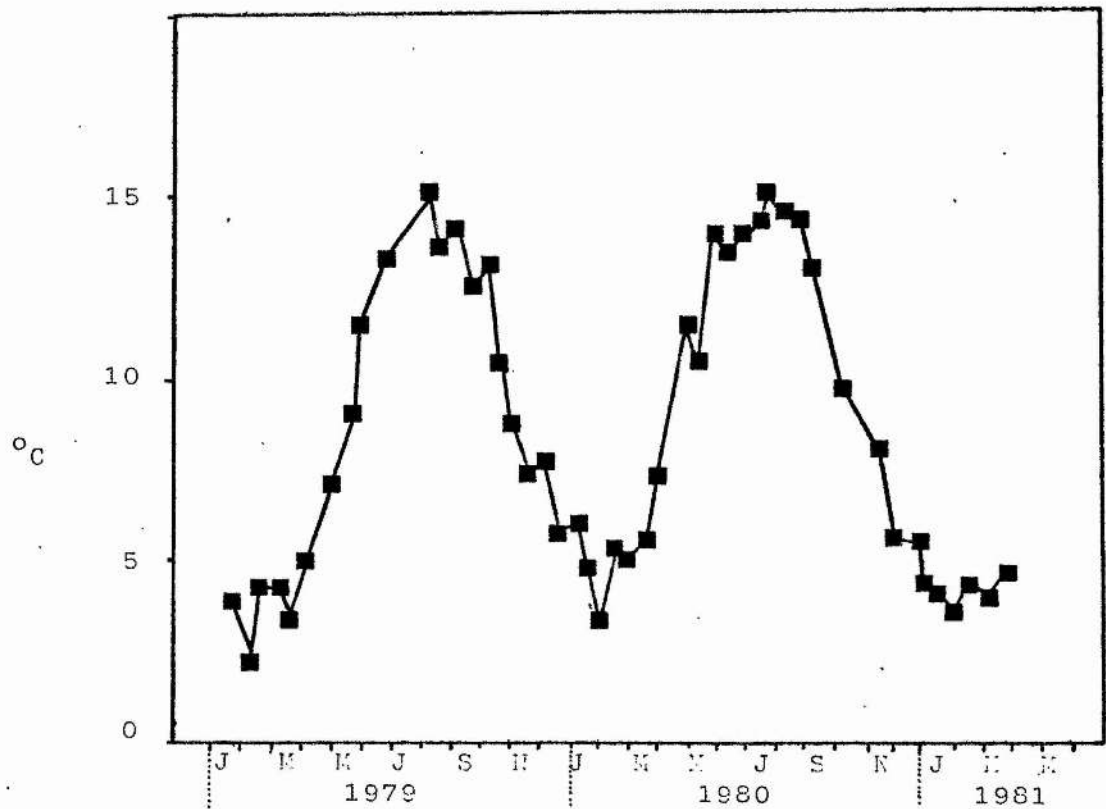
The rapid turnover rate of L. saccharina (possibly resulting from holdfast damage during the marking of plants) meant that few plants were recorded for more than one year. For that reason, the effect of plant age on frond growth rates is not discussed in this species.

ii SEAWATER TEMPERATURE AND SEASONAL GROWTH

Seawater temperature at St. Andrews varies from a maximum of 15 °C in August to a minimum of 2.2-3.5 °C in February (Fig. 3 v). When seawater temperature is considered in relation to seasonal growth rates of L. saccharina (Fig. 3 vi), there is a positive correlation from February-May (not significant) but a negative correlation exists during the rest of the year (June-August, NS; September-November, NS; December-February, $P < 0.10$). The pattern is compressed in L. digitata (Fig. 3 vii) because of a smaller variation between maximum and minimum growth rates. Growth and temperature are positively linearly correlated from February-April ($P < 0.05$), and August-December, and inversely related during December-February (NS), April-June (NS) and June-August ($P < 0.05$).

Although high temperatures may be inhibiting summer growth of Laminaria it is unlikely that temperatures in May (7.5-10 °C) are sufficiently high to be detrimental and cause the decline in linear growth rates after this time. Some other factor is probably limiting growth in May. The seasonal variation in seawater temperature does not offer an adequate explanation for increasing growth rates in January as temperature drops to a minimum in February/March; however, during the autumn and winter, falling temperatures may partially account for declining growth during November and December.

Fig. 3 v. Mean seawater temperature at
St. Andrews recorded fortnightly during 1979-1981.



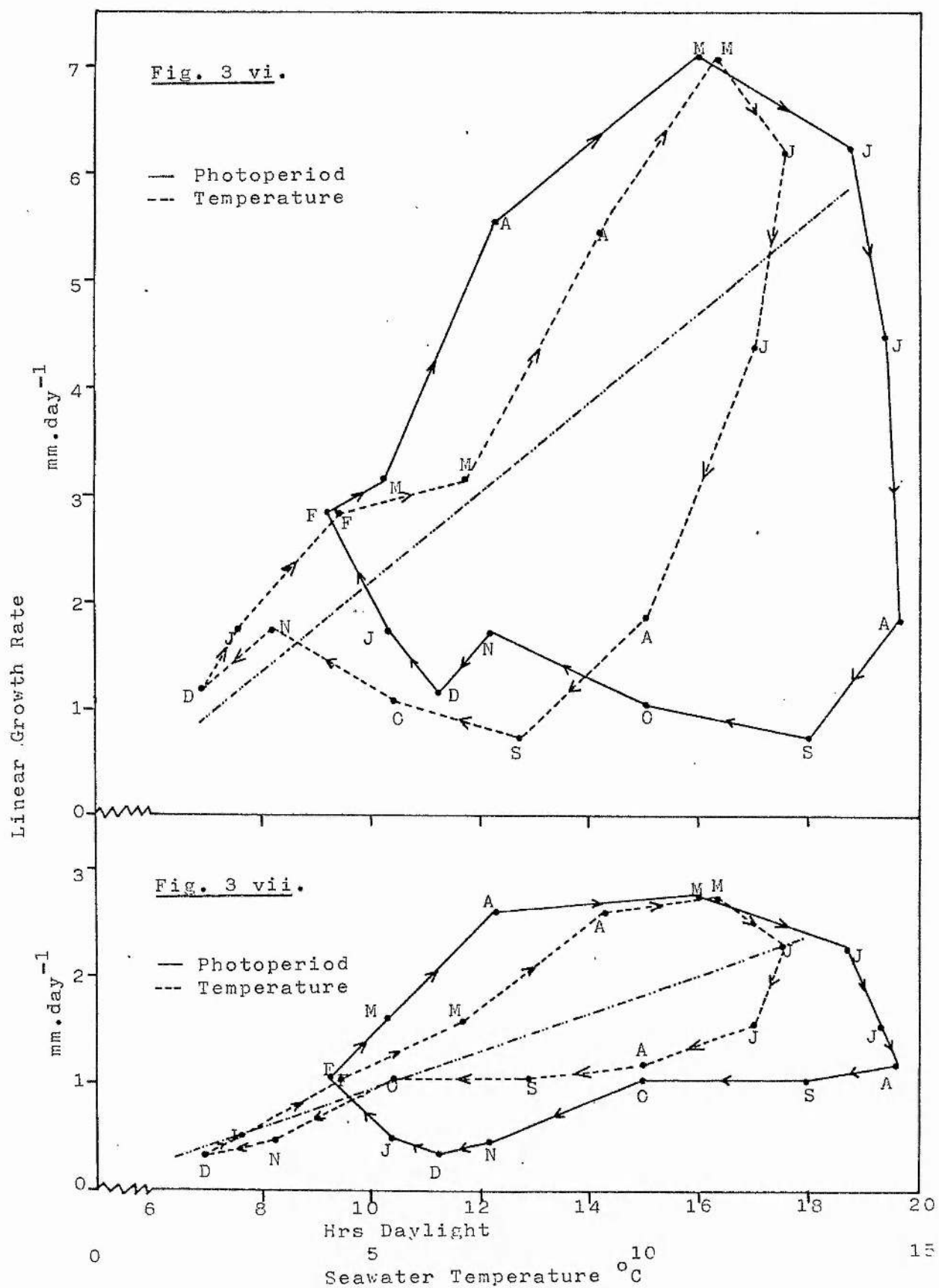


Fig. 3 vi. Relationship of frond growth of *L. saccharina* with photoperiod and seawater temperature.

Fig. 3 vii. Relationship of frond growth of *L. digitata* with photoperiod and seawater temperature.

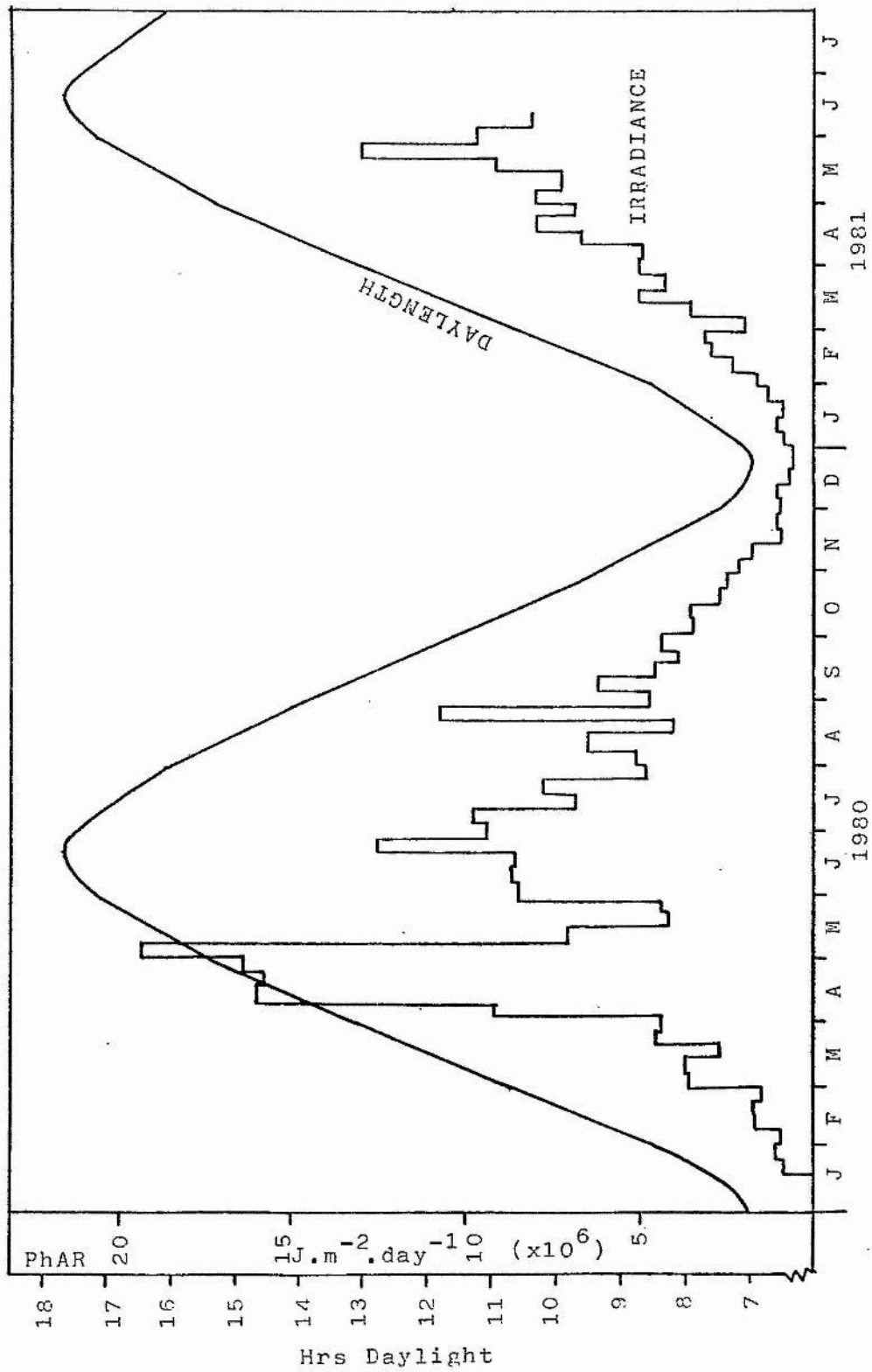
iii LIGHT AND SEASONAL GROWTH

a Photoperiod

Daylength at St. Andrews varies from a maximum of 17 hr 40 mins light (June) to a minimum of 6 hr 56 mins light (December)(Fig. 3 viii). Seasonal growth of L. saccharina (Fig. 3 vi) and L. digitata (Fig. 3 vii) shows a significant positive correlation with photoperiod throughout the whole year (January-December, $P < 0.10$ and $P < 0.02$ for L. saccharina and L. digitata respectively). A significant positive correlation does not necessarily indicate a direct causal relationship but it does suggest that photoperiod may be important in influencing growth of Laminaria (either directly or indirectly) during most of the year.

The minimum daylength for growth in January may be estimated from Figs 3 vi-vii, by continuing basally the line joining December-February to the intersection with the x-axis (ie. at the point where growth is zero). In both L. saccharina and L. digitata this minimum daylength requirement for winter growth is 5 hours; by March/April this has increased to a minimum of approx. 8 hours, and by the peak in May this is much longer because of the effect of temperature. However, it cannot be readily assessed at this time because of other growth limitations which become important, for example, nutrient limiting effects. Even in mid-winter (December) daylength exceeds this minimum requirement, but since growth and photoperiod

Fig. 3 viii. Daylength and irradiance (PhAR. $\text{J.m}^{-2}.\text{day}^{-1} \times 10^6$) measured weekly at St. Andrews during 1980-1981.



are linearly, positively correlated, the short winter days may well be limiting growth of Laminaria.

b Irradiance

Several assumptions are made with regard to the measured irradiance and growth of L. saccharina and L. digitata. In sublittoral algal species (as in L. hyperborea) the amount of light received by the canopy layer during all stages of the tidal cycle may be measured continuously by installation of an underwater light meter on a level with the canopy layer, connected to a pen recorder or digital read-out in the laboratory (for example, see Luning & Dring, 1979). Because of the heterogeneity of the intertidal zone, the amount of usable light received by an alga will depend on its position on the shore, hence the degree of shading by boulders, rock ridges etc, the depth of the water covering the alga and the amount of time it is uncovered during each tidal cycle (there is controversy regarding the ability of Laminaria to maintain normal photosynthetic rates when emersed. Kremer & Schmitz, 1973). Generalisations are difficult for plants which are scattered around the lower intertidal zone. As a result, light measurements were made using a dome solarimeter in an elevated and unshaded position; hence total monthly usable solar irradiance during that month, assuming negligible shading, negligible attenuation with shallow depths of water involved here (predicted from Jerlov, 1951) and the ability to use the

Fig. 3 ix. Relationship between frond growth of L. saccharina and irradiance during 1980.

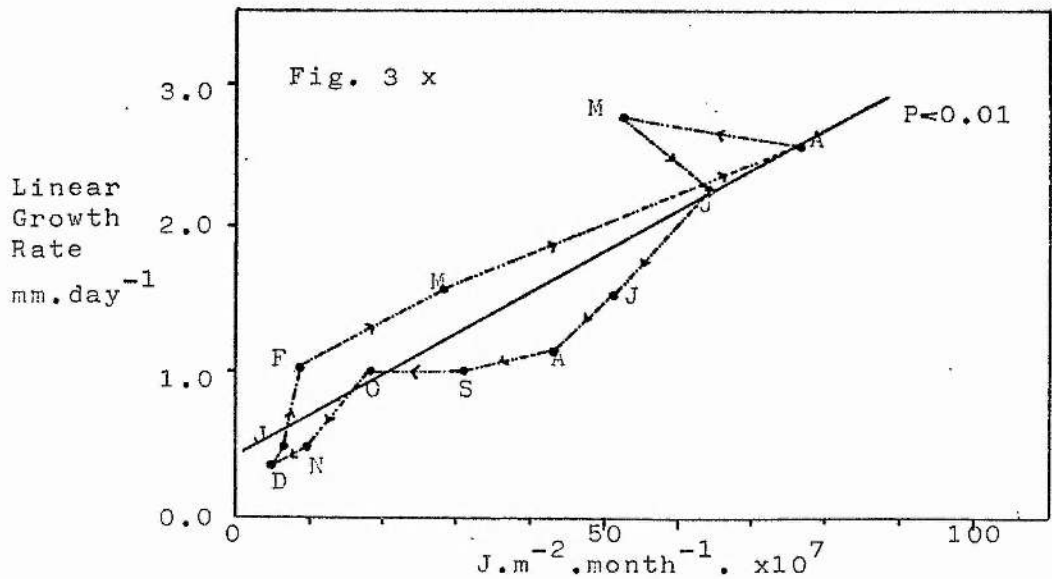
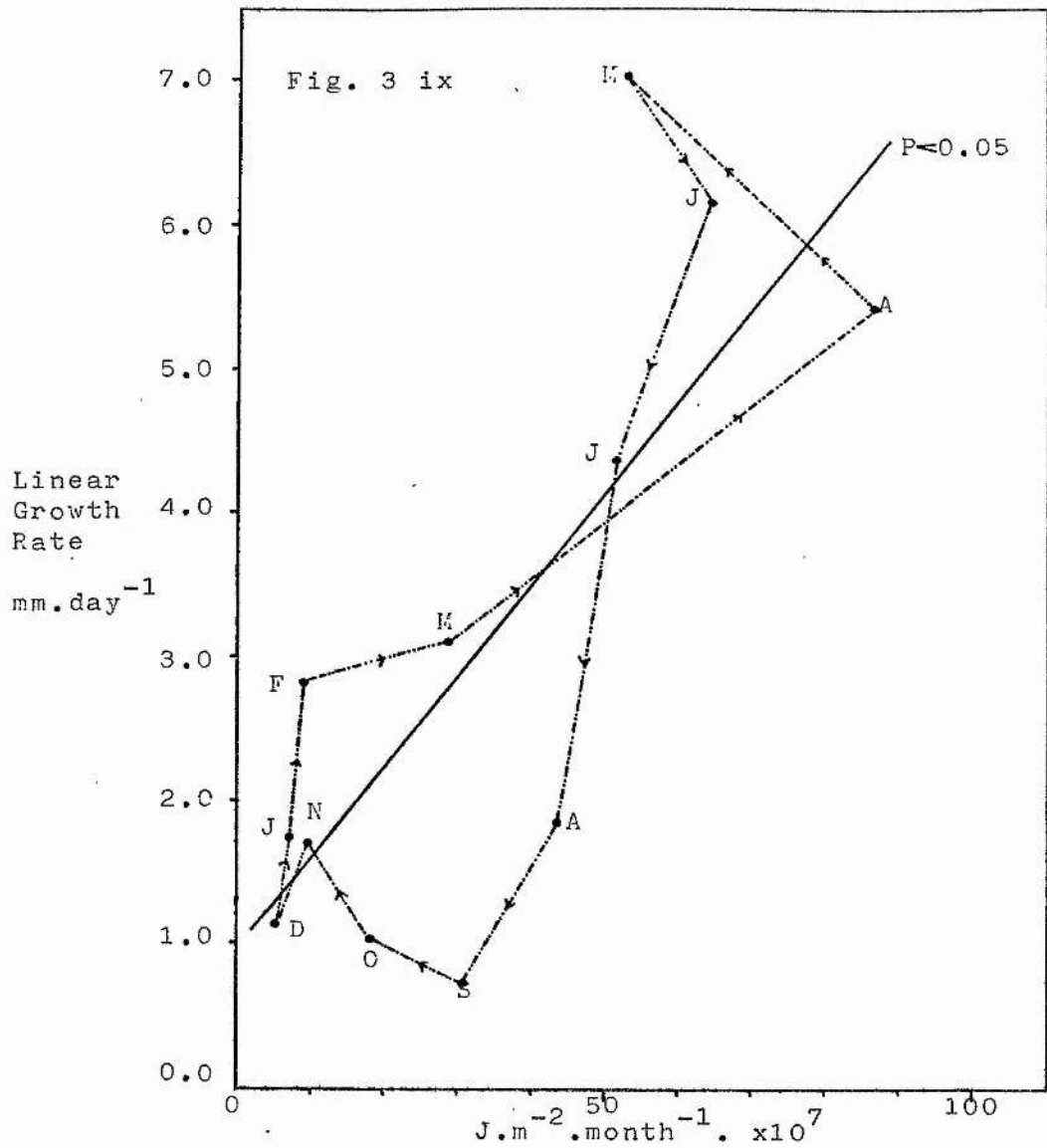


Fig. 3 x. Relationship between frond growth of L. digitata and irradiance during 1980.

available light when emersed.

The seasonal variation in irradiance is shown in Fig. 3 viii. With the exception of the bright, sunny period during April, irradiance increases with increased photoperiod and decreases as photoperiod declines. During 1980, growth of L. saccharina shows a significant positive correlation with irradiance ($P < 0.05$, Fig. 3 ix) although the inverse is true as growth rates increase during September-November. In L. digitata (Fig. 3 x) growth and irradiance also show a significant positive linear correlation ($P < 0.01$) and except during the period April-June this positive relationship is maintained.

Minimum growth, therefore, occurs at a time of low light (November/December) but growth rates begin to increase shortly after daylength and irradiance increase from minimum values in late December. Increasing light may then act as a trigger for the initiation of rapid spring growth rates. Growth rates decline in May as daylength and irradiance (the 1980 pattern of decreasing irradiance from April onwards is probably not the general case every year) are increasing; neither light nor temperature provide an adequate explanation for the declining growth rates in May or for the transient increase in growth rates in September-November.

The effect of temperature and light on growth may be examined by comparing growth rates of Laminaria in 2 months which:

Fig. 3 xi. Relationship between seawater temperature and daylength during 1980 at St. Andrews.

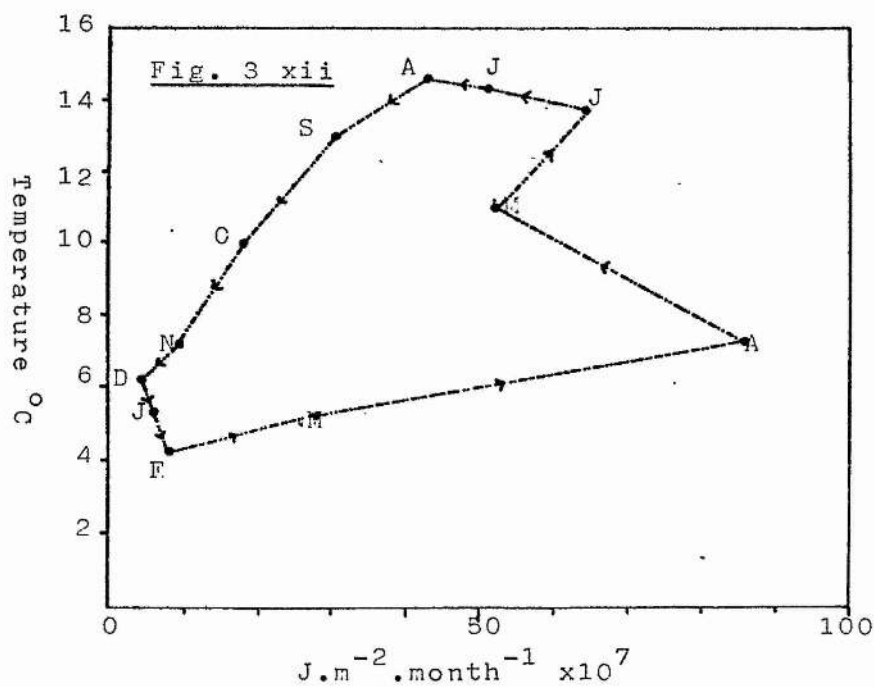
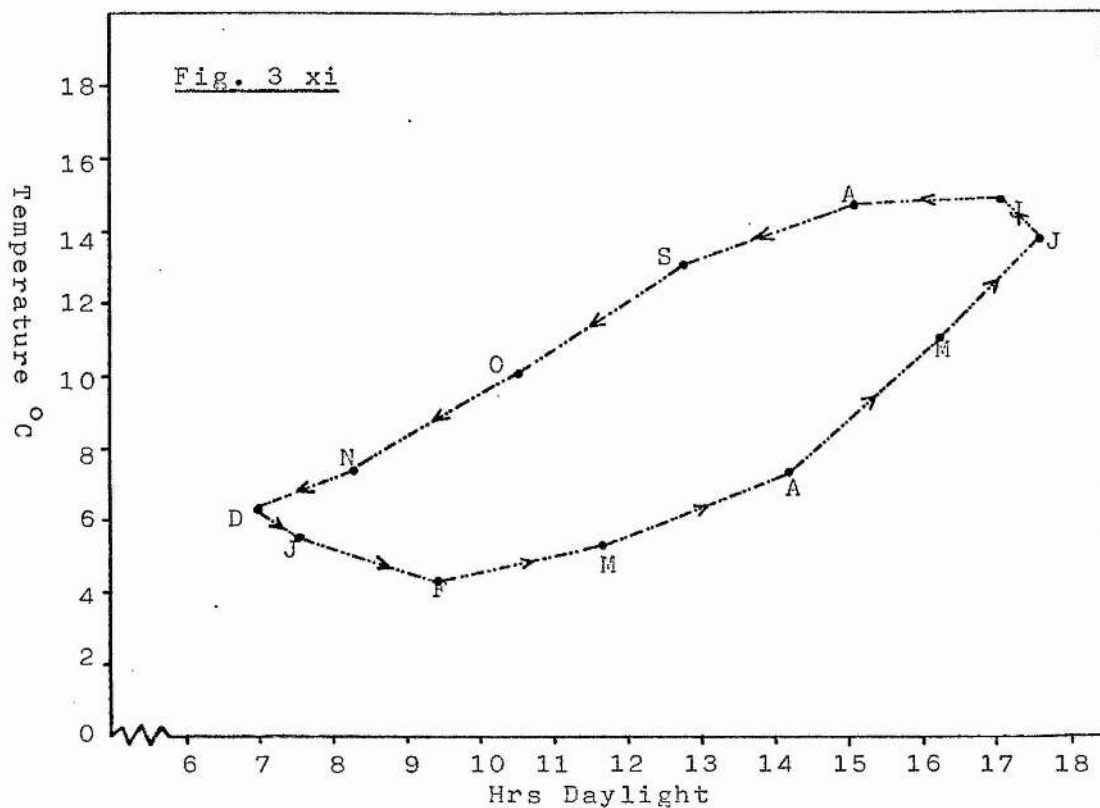


Fig. 3 xii. Relationship between seawater temperature and irradiance during 1980 at St. Andrews.

- i) have similar photoperiods but differ in temperature, and
- ii) have similar temperatures but differ in photoperiod.

Comparing growth rates in March with those in September/October and those in February with October/November (Figs 3 vi-vii) it can be seen that growth rates are significantly higher at the lower temperatures at the beginning of the year. This suggests that higher summer temperatures are inhibitory to growth or that there is a change in algal response to temperature with season (possibly senescence of the frond tissue).

Growth rates are higher when light is higher, as seen when comparing growth rates in May with October and April with November (Figs 3 vi-vii). Light, therefore, appears to be an important external control. However, since temperature and light (photoperiod and irradiance) show a positive linear correlation (Figs 3 xi-xii) for most of the year (except December-February) much of the effect of one factor must also be, either directly or indirectly, the effect of the other factor. There are also other exogenous controls involved during different parts of the year.

Information on the optimum temperatures and photoperiods for growth are required in order to ascertain whether high light or high temperatures limit summer growth of Laminaria and whether light and/or temperature are

limiting growth during the winter.

iv THE EFFECT OF TEMPERATURE ON GROWTH OF
L. SACCHARINA

Small sporophytes of L. saccharina were grown for 14 days in saturating white light (12 hours Light) with high nitrate ($10.5 \mu\text{g-at N.l}^{-1}$) and high phosphate ($3.0 \mu\text{g-at P.l}^{-1}$) on a range of temperatures; 5-30 °C (in 5 °C increments) in November.

Table 3 ii. The effect of temperature on growth of L. saccharina in November. Plants were grown for 14 days with high $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ ($10.5 \mu\text{g-at N.l}^{-1}$: $3.0 \mu\text{g-at P.l}^{-1}$) and a daylength of 12 hours Light. (Mean \pm SE. 3 replicates/treatment)

°C	Growth Rate (mm.day ⁻¹)	% inc. in surface area	% change in dry wt.
5	1.13 \pm 0.48	9.61 \pm 3.63	9.45 \pm 2.75
10	1.71 \pm 0.34	12.03 \pm 3.00	15.72 \pm 4.07
15	2.30 \pm 0.29	21.42 \pm 7.62	16.38 \pm 2.55
20	1.63 \pm 0.46	15.91 \pm 4.67	11.78 \pm 4.33
25	1.42 \pm 0.60*	-	-13.60 \pm 4.69 ⁺
30	-	-	-10.51 \pm 0.46 [±]

* Linear growth rate, mean rate over 0-2 days

+ % change in dry wt. 0-6 days

± % change in dry wt. 0-2 days

Linear growth rates increased to a maximum at 15 °C and declined at temperatures higher than this; the difference between treatments (5-20 °C) was not statistically significant and L. saccharina may be regarded as having a broad temperature optimum (Table 3 ii). Rapid cellular breakdown occurred at 25 ° and 30 °C, within 6 days and 2 days respectively.

Surface area increase and dry weight increase were both greatest at 15 °C, this growth involved both new cell production and cell expansion. Differences between treatments might become more pronounced if the experiment had been extended beyond 14 days.

The optimum temperature for growth (measured by these 3 parameters) appears to be 15 °C, the maximum summer seawater temperature while prolonged periods of low temperatures (5 °C) may be limiting to growth of L. saccharina.

v THE EFFECT OF PHOTOPERIOD ON GROWTH OF L. SACCHARINA

Linear growth rate and surface area showed the greatest increase at 15 hours Light daylength (at near saturating irradiance) (Table 3 iii), whereas dry weight change was greatest at 12 hours Light. Growth dropped markedly at 18 hours light rather than continuing to increase with increasing photoperiod; this may be explained by the wide temperature fluctuations found in this treatment (maximum temperature recorded 23 °C; in all other

Table 3 iii. The effect of photoperiod on growth of L. saccharina in November. Plants were grown for 14 days with high $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ ($10.5 \mu\text{g-at N.l}^{-1}$ and $3.0 \mu\text{g-at P.l}^{-1}$) at 10°C . (Mean \pm SE. 3 replicates/treatment).

Hrs Light	Growth Rate (mm.day ⁻¹)	% inc. in surface area	% change in dry wt.
6	1.07 \pm 0.27	11.52 \pm 1.36	6.03 \pm 1.33
9	1.74 \pm 0.26	6.21 \pm 2.33	8.40 \pm 2.22
12	1.71 \pm 0.34	12.03 \pm 3.00	15.72 \pm 4.07
15	1.83 \pm 0.30	12.29 \pm 2.29	11.50 \pm 3.08
18	1.13 \pm 0.18	10.66 \pm 2.16	4.81 \pm 1.94

treatments the temperature was maintained at $10 \pm 0.5^\circ\text{C}$). The higher temperatures (which are detrimental to L. saccharina, Table 3 ii) may be responsible for the declining growth rates in this 18 hours light treatment. 24 hours light was not carried out due to lack of experimental facilities.

The laboratory experiments show maximum growth at and above 12 hours Light in otherwise favourable conditions. By contrast, in the field daylength exceeds 12 hours Light from March-October, yet growth rates increase to a maximum in May and decline after this probably (see Chapter 6) because of nutrient limitation. Plants in the field are not nutrient limited until May/June since prior to this

the tissue content of N and P increases (see subsequent chapters) so that by inference, temperature and light alone must limit growth until May. Based on data from the November fronds (3 iv and v); temperature appears most to control growth in the spring above 12 hours Light and light most controls in the spring at and below 12 hours photoperiod. However, this March-May increase in growth may indicate that November thalli in the laboratory experiments differ in their responses from young spring tissue and hence, cannot be used as a good measure of spring growth responses. Ideally, the experiments should be repeated at different times of the year, especially on thalli in April/May; unfortunately time was unavailable.

vi THE INITIATION OF NEW FROND GROWTH

Growth rates begin to increase in January as temperature is decreasing to a minimum, but light, intensity and photoperiod are increasing after the shortest day (by the second week in January this represents a daylength inc. of about $\frac{1}{2}$ hour). Data from the November experiments suggest that both temperature and photoperiod might limit growth at this time, but it is proposed that the increasing light (photoperiod and irradiance) may act as a "trigger" for the initiation of spring growth. This hypothesis was tested by investigating the interaction of temperature, photoperiod and nutrients on growth of L. saccharina and of photoperiod and nutrients on L. digitata growth in

Table 3 iv. Linear growth rate (mm.day⁻¹) and % increase in surface area (in parentheses). L. saccharina grown for 14 days in December in saturating white light at photoperiods 7.5 and 17.5 hours Light at 5 and 15 °C and at summer minimum NO₃-N and PO₄-P concentrations (0.5 µg-at N.l⁻¹; 0.3 µg-at P.l⁻¹) and mean winter conc. (10.5 µg-at N.l⁻¹; 3.0 µg-at P.l⁻¹). Mean ± SE with 3 replicates/treatment.

		Temperature °C	
hrs L		5	15
MIN N/P	7.5	1.07 ± 0.07 (2.57 ± 0.67)	1.18 ± 0.03 (8.30 ± 0.63)
	17.5	3.83 ± 0.17 (34.64 ± 0.85)	2.68 ± 0.23 (24.50 ± 1.16)
MAX N/P	7.5	1.17 ± 0.27 (4.81 ± 2.21)	1.73 ± 0.14 (10.44 ± 2.26)
	17.5	2.82 ± 0.52 (24.09 ± 4.76)	2.82 ± 0.49 (19.57 ± 4.98)

Table 3 v. % increase in dry weight of L. saccharina grown in December. (See legend Table 3 iv)

		Temperature °C	
hrs L		5	15
MIN N/P	7.5	6.95 ± 0.72	8.46 ± 2.23
	17.5	26.94 ± 0.19	19.25 ± 1.72
MAX N/P	7.5	7.61 ± 1.56	11.19 ± 0.73
	17.5	21.90 ± 3.74	20.78 ± 5.09

December.

a The interactions of Photoperiod, Temperature and Nutrients on growth of *L. saccharina* in December

Small sporophytes of *L. saccharina* were grown for 14 days (7th - 21st December 1980) at combinations of maximum and minimum temperatures (5 °C and 15 °C); photoperiods (7.5 hours Light and 17.5 hrs L) and nutrients, nitrate and phosphate (0.5 µg-at N.l⁻¹, 0.3 ug-at P.l⁻¹ and 10.5 µg-at N.l⁻¹, 3.0 µg-at P.l⁻¹).

Linear growth rates (Table 3 iv) increased significantly when daylength was increased (at 5 °C:min N/P, P<0.001; 5 °C.max N/P, P<0.02 and at 15 °C.min N/P, P<0.01; 15 °C.max N/P, P<0.10).

Similarly, frond surface area increased significantly with increased photoperiod (Table 3 iv). Temperature had little effect on elongation rates at different photoperiods and nutrients, and at ambient December conditions (7.5 hrs L, max N/P) increasing the temperature did not significantly increase growth rates. The only significant effect of temperature occurs under long days with minimum nutrients (ie. summer conditions) where high temperatures appear detrimental to growth (P<0.02). During short days surface area increase is significantly higher at 15 °C than 5 °C but during long days the reverse is true. Maximum seawater temperatures during the summer may, therefore, be limiting growth of *L. saccharina*.

Nutrients have little effect on elongation rates or surface area change over the short period of the experiment

(14 days); presumably plants under treatments of minimum N and P are utilising stored reserves to maintain growth rates.

The dry weight of plants grown at 17.5 hrs L was significantly higher than at 7.5 hrs L (Table 3 v). During long days, the least increase in dry weight occurred at summer conditions (15 °C with min. N+P), and this is significantly lower than at 5 °C with min. N+P ($P < 0.02$).

When growth was measured using these parameters (linear elongation rate, surface area increase and dry weight increase), light appears to be the most important limiting factor in December. Over 14 days, temperature and nutrients did not significantly affect growth. This lack of an effect of temperature is rather surprising since previously (3 iv) growth was greater at 15 °C than 5 °C; however, the effects of temperature may have been overridden by the dramatic effect of increased photoperiod.

The results suggest that the increasing photoperiod may act as the "trigger" for the initiation of new frond growth. Temperature is relatively unimportant in this respect, although rapid growth, once started might be limited by the low seawater temperatures. External nutrient concentrations are not important in new frond initiation as high internal reserves of N and P allow growth to continue even if the medium is depleted of both nitrate and phosphate. Whether this growth-initiation by a change from shortening to lengthening days was due to the increased

Table 3 vi. % increase in area (Mean \pm SE) of discs of L. digitata cut from the meristem and grown for 20 days in December at 5 °C in saturating white Light at photoperiods, 7.5 and 17.5 hours Light.
(15 discs/Treatment)

		NO ₃ -N ($\mu\text{g-at N.l}^{-1}$)	
		0.5	10.5
PO ₄ -P $\mu\text{g-at P.l}^{-1}$	hrs		
	L		
	7.5	21.54 \pm 1.86	23.40 \pm 0.49
	17.5	67.21 \pm 8.12	75.63 \pm 4.17
4.5	7.5	23.07 \pm 2.58	25.95 \pm 1.04
	17.5	70.72 \pm 7.17	80.80 \pm 6.56

photosynthesis or to true photoperiodism was not directly tested. The mean increment in growth was 2.47 which was similar to that of daylength (2.33) suggesting increased photosynthesis, although the near-saturating irradiances indicate a photoperiodic control. The small effect of varied temperature would be consistent with this, since respiration and carbohydrate reserves might be expected to vary greatly with temperature.

b The interactions of Photoperiod and Nutrients on Growth of Discs of *L. digitata* in December

Discs cut from the meristem of *L. digitata* plants were grown for 20 days (1st - 21st December 1980) at 5 °C at photoperiods (7.5 hrs Light and 17.5 hrs L) in combination with nitrate (at 0.5 and 10.5 $\mu\text{g-at N.l}^{-1}$) and phosphate (at 0.3 and 4.5 $\mu\text{g-at P.l}^{-1}$).

Increasing the photoperiod significantly increases mean disc area (Table 3 vi) where the increment in growth (3.16) was disproportionally larger than that of daylength (2.33) suggesting a photoperiodic control of growth at this time of year. At T=0, disc area=490.94 mm^2 ; after 20 days this had increased to $618.35 \pm 5.11 \text{ mm}^2$ (7.5 hrs L, max N+P) and $887.64 \pm 32.20 \text{ mm}^2$ (17.5 hrs L, max N+P). Nutrients had an effect which was not significant over 20 days.

Using the 3 largest discs/treatment (after 20 days) a small area (4 x 2 mm) of tissue was removed from the centre of each and 20 meristoderm cells (randomly selected) were measured and the mean area estimated. By comparing

Table 3 vii. Ratio of mean cell area to mean disc area of the 3 largest discs/treatment of L. digitata grown for 20 days in December at 5 °C in saturating white light at photoperiods, 7.5 and 17.5 hours Light.

		NO ₃ -N (µg-at N.l ⁻¹)		
hrs L		0.5	10.5	
PO ₄ -P (µg-at P.l ⁻¹)	0.3	7.5	0.0482 ± 0.0006	0.0621 ± 0.0009
		17.5	0.0367 ± 0.0005	0.0423 ± 0.0047
	4.5	7.5	0.0473 ± 0.0013	0.0615 ± 0.0003
		17.5	0.0308 ± 0.0013	0.0242 ± 0.0013

At T=0 ratio is 0.043. Large figures suggest cell enlargement, small figures suggest cell division.

Table 3 viii. % increase in dry weight of discs of L. digitata grown for 20 days in December at 5 °C in saturating white light at photoperiods, 7.5 and 17.5 hrs L. (15 discs/treatment)

		NO ₃ -N (µg-at N.l ⁻¹)			
hrs L		0.5		10.5	
PO ₄ -P (µg-at P.l ⁻¹)	0.3	7.5	12.03 ± 5.30	18.55 ± 1.53	
		17.5	54.42 ± 16.54	35.93 ± 8.28	
	4.5	7.5	19.42 ± 7.74	15.04 ± 4.67	
		17.5	43.84 ± 5.84	44.69 ± 17.29	

the mean cell area with the mean disc area an indication of the method of disc expansion is provided; whether predominantly by cell division (cell area is small relative to disc area) or cell enlargement (cell area is large relative to disc area). At $T=0$, the mean cell area relative to disc area was 0.043; at ambient photoperiods (7.5 hrs L) this ratio increased in all nutrient treatments over 20 days (Table 3 vii), suggesting that increase in disc area occurs predominantly by cell enlargement. Greatest cell enlargement occurs when nitrate is supplied at $10.5 \mu\text{g-at N.l}^{-1}$ (regardless of the phosphate concentration). Since little enlargement occurs at low nitrate concentration, high nitrogen might be important for cell enlargement and high phosphate for cell division at this time of year. However, when the photoperiod was increased, disc growth occurred mainly by rapid cell division, with the smallest ratio (ie. the fastest rate of cell division) when maximum N and P are supplied together. The lowest ratios occur when phosphate concentration is at $4.5 \mu\text{g-at P.l}^{-1}$, again suggesting the possible importance of high phosphate for cell division.

Increasing the photoperiod appears to initiate rapid growth rates by causing rapid cell division; when daylength is short, growth occurs mainly by cell enlargement.

Within 2 days of the start of the experiment, fresh weight increases are significantly higher during long days. After 4 days fresh weight increases linearly with time

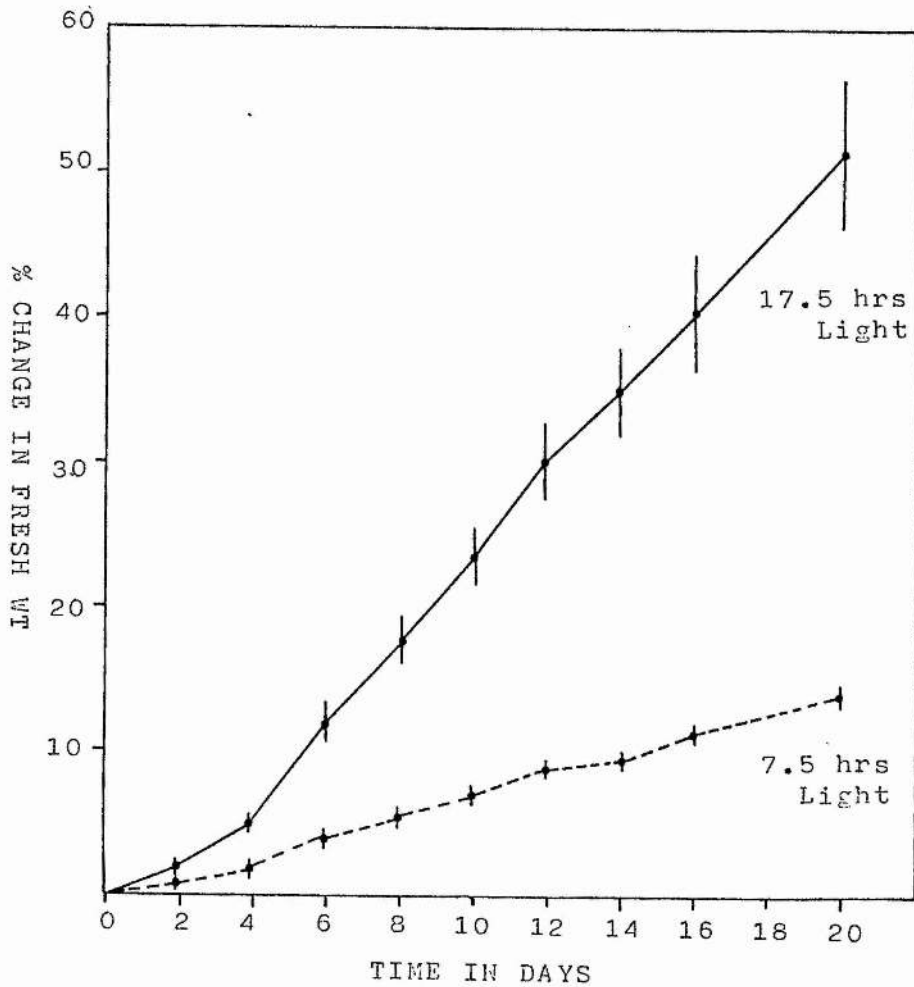


Fig. 3 xiii. % change in fresh weight of discs of *L. digitata* grown for 20 days at 5 °C with 10.5 µg-at N/l (as nitrate) and 4.5 µg-at P/l (phosphate) in saturating white light in December at daylengths 7.5 and 17.5 hrs Light. (15 discs/flask with 3 replicates per treatment. Mean ± SE)

$$17.5 \text{ hrs Light } y=2.64x - 2.11 \quad P < 0.001$$

$$7.5 \text{ hrs Light } y=0.72x - 0.51 \quad P < 0.001$$

($r^2=0.985$, $P<0.001$) at a rate 3.7x greater than the linear increase during short days ($r^2=0.996$, $P<0.001$) (Fig. 3 xiii). After 20 days the dry weight of the discs is significantly higher at 17.5 hrs L compared to 7.5 hrs L (Table 3 viii). The dry weight change was not significantly different for all nutrient combinations within the 2 photoperiods investigated.

Therefore, increasing the photoperiod in December significantly increases growth of discs of L. digitata by initiating rapid cell division. Nutrients have relatively little effect on growth rates in these short-term experiments, probably because internal reserves, which are high in December (Chapters 4+5) may be utilised to support growth during periods of external depletion.

vii THE IMPORTANCE OF STORED RESERVES FOR NEW FROND GROWTH

At a time when the newly initiated frond growth appears to be limited by light and also temperature (deduced from 3 iv and 3 v), photosynthetic production may be inadequate to meet respiratory and growth demands. As a result, stored reserves would necessarily be utilised to maintain rapid spring growth rates.

The importance of stored reserves for new frond growth may be examined in a number of ways:

a Stored reserves are being utilised if the dry weight increase of the frond is considerably less than is expected for the area increase of the frond. This can be

predicted by comparing the dry weight/unit area of the frond prior to rapid spring growth (mid-December) with that once spring growth has begun (the end of January). Since surface area increase was not measured in the field, it is assumed, for the purpose of these calculations, that frond width does not alter over this period.

Assuming that the frond is 50 mm wide and 250 mm in length in December, the increase in frond length of L. digitata from mid-December to the end of January is 16.9 mm (from measurements at St. Andrews).

Actual dry weight of disc (25 mm diameter or 490.9 mm^2) cut from the frond at 20 cm (calculated from carbohydrate analyses) is

Mid-December $97.90 \pm 9.89 \text{ mg}$

End of January $53.03 \pm 3.81 \text{ mg}$

\therefore total dry wt of the frond in December is 2492.9 mg
If production balances utilisation, then the dry wt/unit area will remain the same over the sampling period.

Frond area = width x (original length + length inc)

in January = $50 \text{ mm} \times (250 \text{ mm} + 16.9 \text{ mm})$
 $= 13345 \text{ mm}^2$

If the dry wt remains at $97.90 \text{ mg}/490.9 \text{ mm}^2$

the predicted dry wt of the frond in January is 2661.4 mg

the actual dry wt of the frond in January is 1441.6 mg

The lower than predicted dry weight of the frond might result from

i a loss of stored reserves to the seawater

ii utilisation of stored reserves

iii translocation of reserves away from distal (20 cm) frond tissue. On extrapolation back to the whole frond this would show as an actual decrease in dry weight.

b Carbohydrate content changes relative to size increase. If stored reserves are being utilised to support growth, it may be predicted that endogenous carbohydrates (mannitol and laminarin) would decrease as algal size increases. Although carbohydrate content is measured (Chapter 7) losses to the seawater and frond erosion make calculations of carbohydrate decrease relative to size increase difficult.

However, circumstantial evidence suggests utilisation of stored reserves since mannitol and laminarin content decreases from December to February (Chapter 7) and evidence in the literature indicates that carbohydrates accumulated in the distal frond tissue are translocated basally to support active new frond growth at this time of year (see review, Kain, 1979).

c Removal of stored reserves in October. If stored reserves are important in new frond growth, then removal of most of the accumulated reserves in the autumn (October) would be expected to allow the qualitative response, ie. 'spring' growth begins at the same time, but the actual growth rates achieved by these algae would be significantly reduced.

Growth rates of L. saccharina and L. digitata in situ

Fig. 3 xiv.

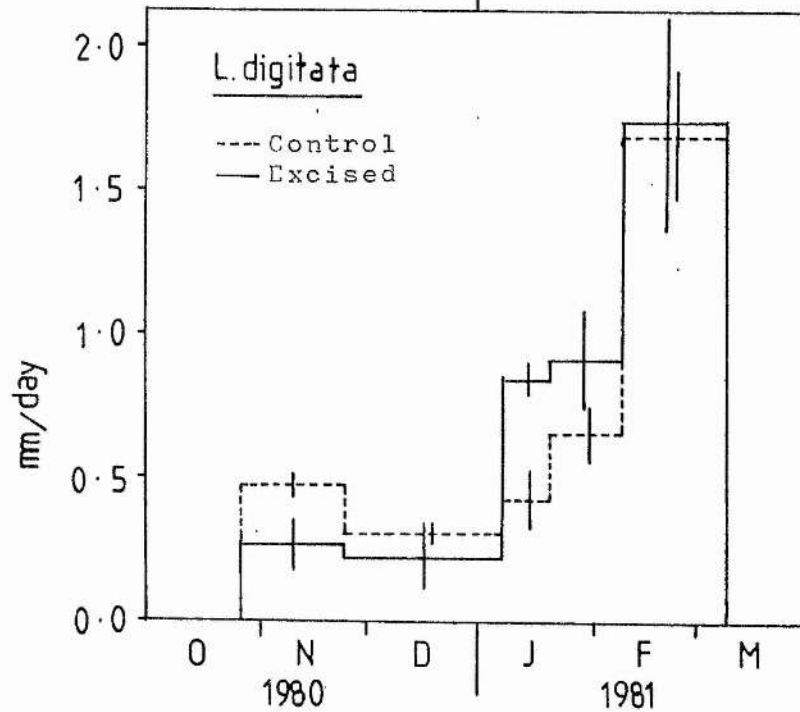
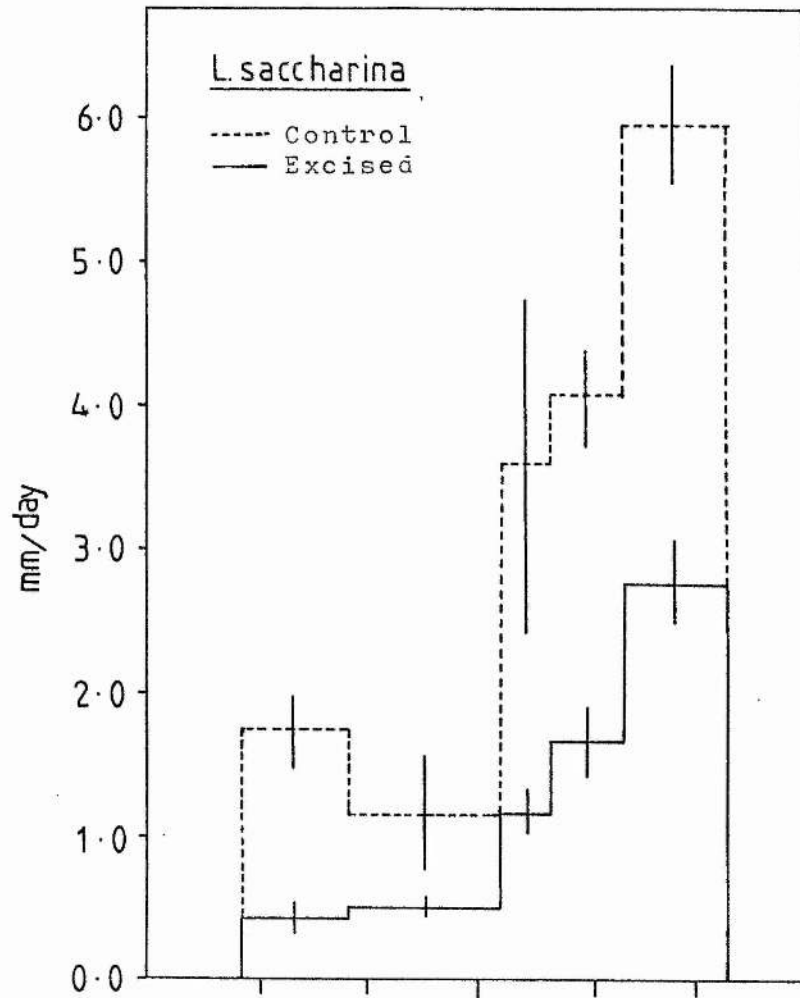


Fig. 3 xv.

Figs. 3 xiv and 3 xv. Growth rates in situ at St. Andrews of L. saccharina (3 xiv) and L. digitata of intact sporophytes (controls) and sporophytes with the frond excised to 10 cm in Oct.

whose fronds had been cut back distally to 10 cm (above the lamina/stipe boundary) in October (thereby removing most of the carbohydrates accumulated during the summer in the mature distal zones), were compared with growth rates of plants marked for seasonal growth measurements (3 i).

In L. saccharina, the qualitative response still occurs, that is, growth rates begin to increase at the same time in both the excised and the control plants (Fig. 3 xiv) but the rates expressed by the excised plants are significantly less than the controls (by March growth rates were $6.45 \pm 0.43 \text{ mm.day}^{-1}$ and $2.81 \pm 0.30 \text{ mm.day}^{-1}$ in the control and excised treatments respectively).

However, in L. digitata (Fig. 3 xv) excision of most of the frond in October did not significantly reduce growth rates compared to the controls. Because of the broader frond of L. digitata, excision to 10 cm leaves a much greater frond area than in the narrower frond of L. saccharina. This extra area of tissue in L. digitata may provide sufficient reserves to support spring growth in this species. This could next be tested by cutting the frond of L. digitata into a narrow band (10 cm in length) in October and comparing spring growth rates with control plants of known frond area. Measurements of relative rather than actual growth rate would probably give a better indication of the importance of frond area and of stored reserves for spring growth.

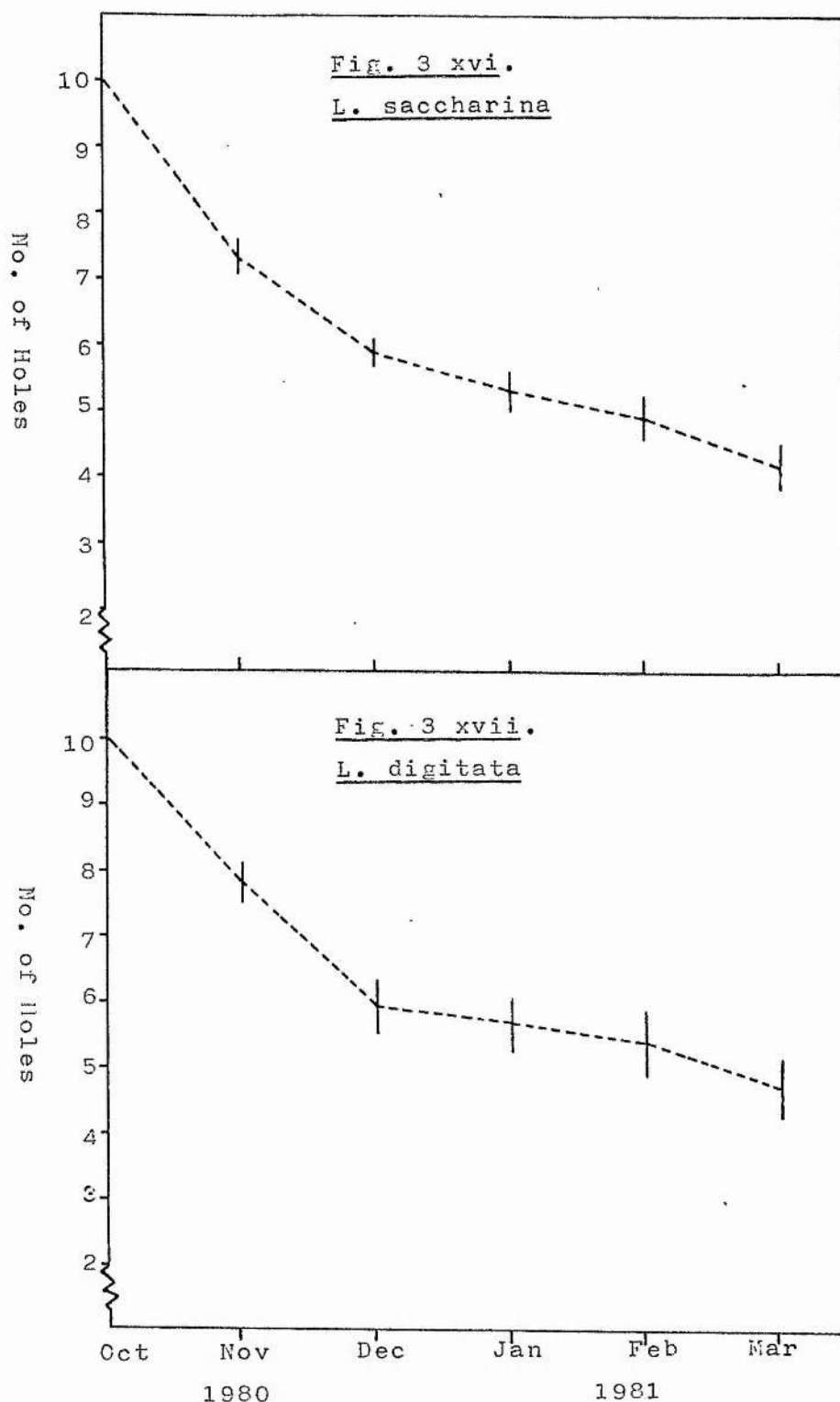
However, it is probable that in both species, stored reserves are necessary for normal spring growth rates.

d Frond Erosion. Continual attrition of the distal frond tissue must remove reserves accumulated during the autumn. The proportion available for utilisation may be seriously reduced by the time spring growth begins in January.

The rate of attrition of the distal frond tissue of L. saccharina and L. digitata in situ was measured by estimating the amount of tissue (by counting the number of punched holes remaining, initially spaced 5 cm apart), remaining at the beginning of each month.

In January, as rapid growth begins, 53% of the frond tissue present in October remains intact in L. saccharina (Fig. 3 xvi) and 57% in L. digitata (Fig. 3 xvii); by March, this is reduced to 41% and 47% respectively. Although the rates of attrition are very variable, depending on the position of plants on the shore and the prevailing weather conditions, it appears that a large proportion (about $\frac{1}{2}$) of the frond tissue present in October (with a high carbohydrate content (Chapter 7)) remains available to the alga by the following January and by March; ie when the new frond is well established there is still a large area of the previous year's frond attached.

Results indicate (3 vii a-d) that stored reserves are probably utilised to support rapid spring growth in both L. saccharina and L. digitata since the loss/decline of carbohydrates probably exceeds photosynthetic production in December/January.



Figs 3 xvi and 3 xvii. Winter loss of summer-formed frond tissue of L. saccharina (3 xvi) and L. digitata (3 xvii) at St. Andrews. In October, 10 holes 5 cm apart were punched in each frond. Loss of holes during the winter measures the erosion of frond tissue.

viii FUNCTIONS OF THE OLD FROND

If Laminaria is partially relying on stored reserves then normal spring growth must necessitate the presence of the previous year's frond, since the old frond would be very important in increasing the storage capacity of the alga (for both carbohydrates and mineral nutrients). If Laminaria is relying on preformed reserves of assimilates then new frond growth may be partially independent of exogenous raw materials and possibly also light, provided the old frond remains attached, and supply of these assimilates from the old frond is adequate to support growth. In order to supply adequate reserves the old frond may be important in increasing the area for photosynthesis and for nutrient uptake both in the previous season and currently, in addition to acting as a reserve for carbohydrates and mineral nutrients.

In January, once spring growth has been initiated, experiments were carried out on L. saccharina to compare growth of the new frond with the old frond attached, with growth of the new frond alone (old frond excised, but holdfast and stipe still present). The results were examined in relation to the possible functions of the old frond mentioned above.

a Is new frond Growth Independent of Exogenous Chemical Raw Materials?

Growth, as linear growth and surface area increase (Table 3 ix), of the new frond of L. saccharina is the

Table 3 ix. Linear growth rate (mm.day^{-1}) and % inc.

in surface area of the new frond of L. saccharina when the old frond is excised (-OF) and when the old frond is attached (+OF). Plants were grown for 20 days at 5 °C in January at ambient photoperiod (7.5 hrs L) in ambient seawater ($12.0 \mu\text{g-at N.l}^{-1}$; $2.5 \mu\text{g-at P.l}^{-1}$) and in nitrate depleted seawater ($0.5 \mu\text{g-at N.l}^{-1}$; $2.5 \mu\text{g-at P.l}^{-1}$). The experiment was repeated (b) for growth in ambient seawater (see above) and in phosphate depleted seawater ($12.0 \mu\text{g-at N.l}^{-1}$; $0.3 \mu\text{g-at P.l}^{-1}$).

Mean \pm SE with 3 replicates/treatment.

(a)	-OF	+OF	Significance
Amb	0.99 ± 0.10	2.38 ± 0.28	$P < 0.01$
sw	(35.96 ± 3.64)	(97.12 ± 10.03)	$P < 0.01$
-N	0.93 ± 0.10	2.82 ± 0.37	$P < 0.01$
	(28.43 ± 1.40)	(95.58 ± 14.17)	$P < 0.01$
(b)	-OF	+OF	Significance
Amb	1.89 ± 0.11	3.13 ± 0.22	$P < 0.01$
sw	(103.00 ± 16.32)	(158.29 ± 23.88)	NS
-P	1.38 ± 0.29	2.41 ± 0.20	$P < 0.05$
	(81.04 ± 17.48)	(164.13 ± 15.64)	$P < 0.05$

Amb sw = Ambient seawater

Surface area increase (%) = figures in parentheses

same in ambient and depleted (both -N and -P) seawater if, and only if, the old frond is present. Therefore, in January, over 20 days, in normal field conditions (ie. with the old frond attached) new frond growth can be independent of the external nutrient concentration. In addition, high carbohydrate reserves in December/January (Chapter 7) may indicate that, at this time, the alga can be independent of all exogenous chemical raw materials. The independence of new frond growth with light was not tested but Luning (1969) showed that even in complete darkness L. hyperborea was capable of producing a small new frond by relying on reserves of the old frond.

b The old frond functioning as a storage organ for both carbohydrates and mineral nutrients.

The results (Table 3 ix) show that once spring growth is initiated the presence of the old frond is necessary for normal spring growth rates (linear increase and surface area increase). Dry weight change is also significantly greater when the old frond is present than when it is absent (Table 3 xi).

Growth rates are independent of the external nitrate and phosphate concentration when the old frond is present. In the depleted seawater, the alga must be relying on internal N and/or P reserves to maintain rapid growth rates. When the old frond is excised, growth of the new frond is slower in depleted than in ambient seawater, but this effect is not significant over 20 days since the new frond

Table 3 x. Linear growth rates ($\text{mm} \cdot \text{day}^{-1}$) and % inc. in surface area (in parentheses) of the new frond of *L. saccharina* when the old frond is excised (-OF) and when the old frond is present (+OF) in ambient seawater ($12.0 \mu\text{g-at N} \cdot \text{l}^{-1}$: $2.5 \mu\text{g-at P} \cdot \text{l}^{-1}$), nitrate-depleted seawater (-N) ($0.5 \mu\text{g-at N} \cdot \text{l}^{-1}$), x2N ($24.0 \mu\text{g-at N} \cdot \text{l}^{-1}$) and x3N ($36.0 \mu\text{g-at N} \cdot \text{l}^{-1}$). Plants were grown for 20 days in January at 5°C at ambient photoperiod (7.5 hrs L). Mean \pm SE with 3 replicates/treatment. All treatments have phosphate at $2.5 \mu\text{g-at P} \cdot \text{l}^{-1}$.

	-OF	+OF	Significance
Amb	0.99 ± 0.10	2.38 ± 0.28	$P < 0.01$
sw	(35.96 ± 3.64)	(97.12 ± 10.03)	$P < 0.01$
-N	0.93 ± 0.10	2.82 ± 0.37	$P < 0.01$
	(28.43 ± 1.40)	(95.58 ± 14.17)	$P < 0.01$
X2N	1.14 ± 0.12	-	-
	(43.44 ± 1.36)	-	-
X3N	1.24 ± 0.27	-	-
	(51.00 ± 6.26)	-	-

Table 3 xi. % increase in dry weight (in parentheses) and % change in mannitol content of the new frond of *L. saccharina*. See legend to Table 3 x. (Mannitol content, - denotes loss to the tissue) NF = New frond, OF = Old frond

	-OF	NF	+CF	OF
Amb	(27.63 ± 4.33)	(67.14 ± 6.00)	(11.12 ± 1.76)	
sw	296.05 ± 46.50	286.97 ± 54.82	-31.93 ± 6.41	
		0		
-N	(26.56 ± 1.33)	(69.01 ± 14.08)	(17.52 ± 1.66)	
	260.49 ± 69.71	390.51 ± 41.12	-41.57 ± 7.00	
X2N	(33.31 ± 2.97)	-	-	
	-57.35 ± 12.92	-	-	
X3N	(34.52 ± 6.97)	-	-	
	138.46 ± 10.57	-	-	

Table 3 xii. Internal N content of the new frond and the old frond of L. saccharina when the old frond is excised (-OF) and when the old frond is present (+OF). See legend to Table 3 x. Total N content in $\mu\text{g N} \cdot 100 \text{ mg dry wt}^{-1}$.

		Total-N
T=0	NF	572.11 \pm 84.48
T=20		
Amb sw	NF (+OF)	825.20 \pm 53.60
	NF (-OF)	583.73 \pm 17.66
-N	NF (+OF)	759.33 \pm 24.45
	NF (-OF)	505.49 \pm 19.03
X2N	NF (-OF)	617.91 \pm 5.26
X3N	NF (-OF)	669.39 \pm 4.07
T=0	OF	644.34 \pm 45.20
T=20		
Amb sw	OF	997.29 \pm 164.91
-N	OF	735.35 \pm 58.65

maintains growth by utilising its own stored reserves (see Tables 3 xii and 3 xv).

The effect of nitrate is considered first (see ambient seawater and N-depleted seawater only on Table 3 xii). The total N content of the new frond is significantly lower when the old frond is excised than when it is attached. In N-depleted seawater (-OF) it is the inorganic N fraction which shows the greatest decline, presumably as it is utilised for organic-N synthesis, but within 20 days, all 3 nitrogen fractions have fallen. (Measurements in Table 3 xii are relative to dry weight). As a result the total N content of the new frond (-OF) after 20 days is significantly less than at T=0). It appears to be the protein fraction which is affected primarily by the nitrogen supply: when there is adequate N available (ambient seawater +OF) a large proportion of the N is channelled into protein synthesis, but when the immediate supply is reduced (ie. the external concentration remains the same but already accumulated N is removed by excision of the old frond) the synthesis of protein is decreased and all 3 N forms decrease. The old frond is, therefore, necessary in supplying an immediate, utilisable store of nitrogen as inorganic, organic and protein N.

Similarly, with regard to phosphate (Table 3 xv. see ambient seawater and P-depleted seawater only), when the old frond is attached rapid growth rates are maintained in P-depleted seawater as internal reserves are utilised.

Table 3 xiii. Linear growth rates (mm.day^{-1}) and % increase in surface area (in parentheses) of the new frond of *L. saccharina* when the old frond is excised (-OF) and when the old frond is present (+OF) in ambient seawater ($12.0 \mu\text{g-at N.l}^{-1}$; $2.5 \mu\text{g-at P.l}^{-1}$), phosphate-depleted seawater (-P) ($0.3 \mu\text{g-at P.l}^{-1}$), X2P ($5.0 \mu\text{g-at P.l}^{-1}$) and X3P ($7.5 \mu\text{g-at P.l}^{-1}$). All treatments have nitrate at $12.0 \mu\text{g-at N.l}^{-1}$. Plants were grown for 20 days in January at 5°C at ambient photoperiod (7.5 hrs L). Mean \pm SE with 3 replicates/treatment.

	-OF	+OF	Significance
Amb	1.89 ± 0.11	3.13 ± 0.22	$P < 0.01$
sw	(103.00 ± 16.32)	(158.29 ± 23.88)	NS
-P	1.38 ± 0.29	2.41 ± 0.20	$P < 0.05$
	(81.04 ± 17.48)	(164.13 ± 15.64)	$P < 0.05$
X2P	1.73 ± 0.15	-	-
	(87.36 ± 15.27)	-	-
X3P	2.08 ± 0.15	-	-
	(103.84 ± 15.21)	-	-

Table 3 xiv. % increase in dry weight (in parentheses) and % change in mannitol content of the new frond of *L. saccharina*. See legend to Table 3 xiii.
NF = New frond, OF = Old frond

	-OF	NF	+OF	OF
Amb	(58.25 ± 6.31)	(103.47 ± 15.76)		(12.25 ± 1.98)
sw	200.10 ± 26.09	391.23 ± 81.80		178.95 ± 3.87
-P	(50.18 ± 11.89)	(78.87 ± 12.88)		(13.81 ± 3.68)
	126.58 ± 23.91	718.70 ± 44.34		186.32 ± 20.35
X2P	(50.16 ± 9.50)	-		-
	280.24 ± 17.53	-		-
X3P	(67.27 ± 7.03)	-		-
	440.67 ± 26.35	-		-

Table 3 xv. Internal P content ($\mu\text{g P.100 mg dry wt}^{-1}$)
of the new frond and the old frond of L. saccharina.
See legend to Table 3 xiii.

	-CF	NF	+OF	OF
T=0	349.89 \pm 17.91	349.89 \pm 17.91	645.79 \pm 12.20	
T=20				
Amb	212.22 \pm 40.07	373.52 \pm 117.79	663.51 \pm 44.31	
sw				
-P	221.33 \pm 40.71	330.04 \pm 33.66	472.80 \pm 46.59	
X2P	460.27 \pm 61.09	-	-	
X3P	525.13 \pm 40.55	-	-	

Mannitol production (in a longitudinal strip of tissue from the midline of the new frond and the old frond) increases significantly over 20 days in the new tissue, whether the old frond is attached or excised (Tables 3 xi and 3 xiv). Mannitol content of the old frond declines markedly, possibly due to basipetal translocation, respiratory losses and perhaps losses to the seawater. In situ mannitol production of both old and new tissue cannot keep pace with the growth requirements of the new tissue and continues to decline to a minimum in March (Chapter 7). Mannitol content increases significantly in the new frond when the old frond is attached and also in the new frond only, hence the increase does not simply result from basipetal translocation, and there is a net mannitol production by the new tissue. Hence irradiance, in situ must limit carbohydrate production. If this is the case, the old frond must be required as a reserve of carbohydrate as well as providing an increases area for photosynthesis, whose products might add to those translocated to the new frond and perhaps also provide energy for the translocation process. An increased area would be an advantage particularly during periods when irradiance is above the light compensation point for photosynthesis but would help even if the light intensity is below compensation point.

The old, non-growing frond, therefore, is important for new frond growth in providing a large reserve pool of translocatable N-compounds, P-compounds and carbohydrates

(particularly mannitol) before it decays or is torn off; as such it resembles a deciduous leaf in early autumn, exporting nutrients.

c The old frond providing an increased area for mineral (N and P) uptake. The old frond may also have a role in providing an increased area for nitrate and phosphate uptake both in the spring and in the previous autumn. The importance of this role was estimated by

- i comparing the N or P content of the new frond/old frond when the old frond is attached or excised, and
- ii by increasing the external N or P concentration in treatments with new frond only (ie. -OF) to investigate uptake capability of the isolated new frond.

i The total N content (Table 3 xii) of the new frond with the old frond attached is significantly higher than when the old frond has been excised, in both ambient and N-depleted seawater. The total N content of the attached old frond also increases, indicating uptake of N from the medium. The results suggest that most of the N-uptake occurs in the old frond, followed by translocation basally, resulting in an increases N content of the new frond when attached to the old frond. Similarly, with the tissue P-content (Table 3 xv) the presence of the old frond results in a significantly increased P-content of the new frond, in comparison to new frond only (-CF).

It, therefore, appears that the old frond is important

in providing an increased area for continued N and P uptake concurrent with spring growth of the new frond, at a period when available inorganic N and P concentrations in the seawater are high.

ii The isolated new frond differs in its uptake capability of N and P. The total N-content of the new frond (-OF) is raised only slightly above the "ambient" and "N-depleted" treatments when the external nitrate concentration is doubled or trebled (Table 3 xii). The new frond appears to have only a limited capacity for nitrate uptake. However, the tissue P content (Table 3 xv) increases approximately in proportion to the supply concentration. The actual change in P content ($\mu\text{g P}/100 \text{ mg dry wt} \times \text{dry wt increase}$) at x2P is 1.87 and at x3P, 2.86 times greater than at ambient phosphate concentrations. The actively growing tissue, therefore has a lower capacity for N uptake than the older tissue, but this is not so for phosphate uptake. The old frond therefore has a quantitatively important role in increasing the area for N uptake in situ in the winter and to a lesser extent in increasing the area for phosphate uptake, in the latter case the young tissue itself provides more of the P-compounds required for growth.

d The old frond providing an increased area for photosynthesis. In the laboratory experiments (Tables 3 xi and 3 xiv) the new frond of L. saccharina has a significantly higher mannitol content when the old frond is attached than when it is excised. This may be

accounted for both in terms of translocation of mannitol from the old to the new frond and of increased photosynthetic production by the new frond itself because increased new growth when the old frond is attached resulted in an increased area for photosynthesis. In the old frond, however, the results are rather variable; on the one hand translocation appears to be very much greater than photosynthetic input (Table 3 xi), hence mannitol content declines, in the other instance, the mannitol content of the old frond also increases significantly over the course of the experiment (Table 3 xiv). It is, therefore, difficult to distinguish the effects of translocation from those of photosynthetic production of the old frond. In addition, optimal conditions for new frond growth may differ from those for old frond (and new frond) photosynthesis, hence translocation controlled by growth demand (see chapter 7) might be temporally separated from photosynthetic production by the old and new frond tissue. The importance of this role of increasing the surface area for photosynthesis is, therefore, not clear but as indicated in (b) above, irradiance in situ is likely to be limiting carbohydrate production, and the old frond probably acts more as a reserve of previously synthesised carbohydrate than providing an increased area for current photosynthesis. However, an increased area for photosynthesis would be an advantage during periods when irradiance is sufficiently high to be above the light compensation point for photosynthesis. Further information is required on the actual

amount of light received in situ in order to estimate the importance of the old frond in this role.

The old frond is essential to growth of the new frond during the spring in L. saccharina in providing carbohydrates at a time of probable light limitation in situ and in increasing the area for nutrient uptake, in addition, to providing reserves of N and P. Growth rates of isolated new fronds cannot be enhanced to the rates obtained when the old frond is present, by the addition separately of high concentrations of nitrate or of phosphate to the medium. However, the effects of simultaneous enrichment with high NO_3 and PO_4 requires testing. Although isolated new fronds can photosynthesize mannitol and absorb exogenous nitrate and phosphate, not even the simultaneous supply of high light, CO_2 and enriched nitrate or phosphate levels externally can raise the growth rates of isolated new fronds to those of new fronds with attached old fronds. This suggests that the old frond may have additional roles, not yet considered, of translocating vitamins and growth-promoting substances to the new frond to produce the high spring growth rates.

DISCUSSION

Using the punched hole technique of Parke (1948) the seasonal pattern of growth of L. digitata and L. saccharina at the 4 sampling sites has been described. In both species, frond elongation rates begin to increase in January to a peak in May before declining to low rates of

growth during the summer, autumn and winter. The division into a "period of rapid growth" (January-June) and one of "slow growth" (July onwards) (Parke, 1948) is in agreement with other worker's findings for L. digitata (Sundene, 1964; Cosson, 1967; Perez, 1971; Mann, 1972b) and L. saccharina (Parke, 1948; Johnston et al, 1977). In L. hyperborea this distinction between the slow and rapid growth periods is very marked and the total increase in length during the slow growth period is less than 5 cm compared to a growth rate of about 0.9 cm.day^{-1} in April (Kain, 1976).

This capacity for late winter growth rising to a spring maximum and then declining during the summer has attracted attention, with attempts to explain this pattern in terms of endogenous and exogenous controls. The importance of light and temperature are examined here.

Seasonal changes in seawater temperature alone do not provide an adequate explanation for the observed growth patterns beyond the suggestion that low temperatures may limit winter growth and that high summer temperatures may be detrimental to Laminaria. Experimental data on the growth responses of L. saccharina to various temperatures suggests that the minimum temperatures experienced in situ may well be limiting growth in winter. Thus, growth should be most limited in February when seawater temperature is at a minimum; however, the field data shows rapidly increasing growth rates at this time and temperature limit-

ation must be overridden by some other factor, possibly light. In agreement with the November thalli experiments, Fortes and Luning (1980) showed that L. saccharina has a broad temperature optimum of 10-15 °C, and that higher temperatures (20 °C and upwards) were detrimental. The decline in growth rates in May of L. saccharina and L. digitata is unlikely to be an effect of increasing temperature (May seawater temperature is only 7.5 - 10 °C) and since, in situ, temperatures rarely exceed 15 °C the summer decrease in growth rates cannot be a simple response to temperature. However, in one species of Laminaria, L. japonica the summer decline in growth rates is directly attributed to high summer seawater temperatures exceeding 20 °C (Tseng et al, 1957).

Similarly, the decline in growth in May cannot adequately be explained as a response to light (either photoperiod or irradiance) unless high light, in combination with higher seawater temperatures is inhibitory. The November thalli experiments on L. saccharina suggest growth to be saturated at 12 hours Light, but the inconclusiveness of this experiment and that regarding temperature growth responses indicate a changing algal response with season (and hence with age of the frond) and that Fortes & Luning's (1980) data showing increased growth with increasing photoperiod (up to 24 hours light) probably gives the true response of Laminaria in Spring/summer (when the algae were collected and tested for this work). Photoperiod

would, therefore, not be expected to cause a slowing of growth before daylength had reached a maximum. Growth is significantly correlated with irradiance during the year, but the 1980 irradiance maximum in April is not typical (maximum usually coincides with the maximum day-length) and hence the consistent decline in Laminaria growth rates in May is not simply a response to decreasing irradiance. However, Fortes & Luning (1980) found irradiances of $250 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (equivalent to $50 \text{ W} \cdot \text{m}^{-2}$) to be detrimental to growth of L. saccharina. This irradiance is exceeded from mid-March to the beginning of October at St. Andrews, and if this figure is correct, growth of L. saccharina must be limited for a large proportion of this time. Perhaps a more realistic figure is provided by Drew (1974) where irradiances of $250\text{--}300 \text{ W} \cdot \text{m}^{-2}$ were not inhibitory to L. digitata. More work is required to clarify this matter, however.

The decline in growth rates in May cannot be adequately explained in terms of seawater temperature and light (photoperiod or irradiance). Early in the year, January-May, temperature and light must be the 2 most important external factors controlling growth of Laminaria since nutrient effects are unlikely to be significant at a time of maximum external nutrient concentrations and high and increasing tissue content (see subsequent chapters).

From the November data, growth is saturated at photoperiods of 12 hours light and over, hence it would be

reasonable to suggest that light most controls growth in the spring at less than 12 hours light and that once the light requirement is met, growth would then be most controlled by temperature. Mineral limitation would only operate from late spring as the temperature rises. However, day-length exceeds 12 hours light from mid-March, but seawater temperature is close to its minimum and a change to temperature controls on growth would probably cause a slowing or a decline in growth rates, rising only as temperature increased during the summer. The continued rapid rise during March-May suggests that the alga continues to respond to increasing photoperiod and temperature simply interacts with light at this time. This again emphasises the disadvantages of using experimental responses of the old thallus in November to interpret new spring growth responses.

New frond growth is first evident during the 2nd week in January in both L. digitata and L. saccharina; at a time when both temperature and light (photoperiod and irradiance) are likely to be limiting. The factors which trigger this growth increase have not clearly been ascertained in these 2 species but increasing daylength after the shortest day may be important in this respect. Chapman & Craigie (1977) found that seasonal changes in growth rate of L. longicruris resulted from rising nitrate concentration of the seawater and the growth rates increased as ambient nitrate increased in the autumn. Although nitrate concentration may play a significant role in

controlling seasonal growth in L. saccharina and L. digitata, it does not have such an obvious direct effect on growth and is not solely responsible for triggering the initiation of new spring growth in these species. L. saccharina and L. digitata both show an increase in growth rates as nitrate (and phosphate) increases in the autumn (September/October)(Chapters 4 and 5) but this effect is shortlived despite the continued nutrient increase and other factor(s) limit growth up until January when new spring growth begins. The interaction of photoperiod, temperature and nutrients suggests that light is the most important limiting factor in December. Temperature, which had an effect which was not significant at the number of replicates used may also have a limiting effect which operates over a longer period than the 14 day duration of the experiment. Laminaria growth rates, therefore, decrease to a minimum in November/December as light becomes more limiting and as temperature decreases.

The hypothesis that spring growth is initiated by increasing light after the shortest day is supported by evidence from L. saccharina (3 iva) and the subsequent experiment with discs of L. digitata (3 ivb). Increasing the photoperiod in December (before the shortest day) resulted in rapid growth by "switching-on" cell division where previously, growth under ambient photoperiods was predominantly by cell enlargement. The laboratory experiments were carried out at near saturating light intensities

(Drew, pers comm. where light saturation for photosynthesis was $50-100 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in both L. saccharina and L. digitata) yet there is still a large effect of photoperiod, suggesting that this is a true photoperiodic trigger rather than an effect of an increase in total light. This could best be tested by night-break type experiments, producing long days artificially by night breaks during short days. If there is a true photoperiod control, rapid spring growth would be initiated under short days + night break but not under short days themselves.

There appears to have been a qualitative change in the algal response to light between November and December. In November, increasing the photoperiod resulted in increasing growth up to 12 hours light but this growth increase was not significantly greater than growth under the shorter days, and there was not the dramatic effect which is apparent when photoperiod was increased in December. This changing response at the end of the year lends weight to the suggestion that the frond may undergo "senescence" as it ages during the year (the term is used guardedly here since this effect may not be as irreversible as senescence implies). This aspect is pursued further in subsequent chapters.

Increasing photoperiod may act as the "triggering mechanism" for cell division to be "switched-on" and for growth rates to increase, yet the newly initiated growth is limited at the time by total irradiance and temperature,

and ambient photoperiod is probably inadequate for the production of sufficient organic materials (this is the case in L. hyperborea, Luning, 1971). As a result, the alga must rely on stored reserves as well to meet the total new frond growth requirements. The utilisation of stored reserves is indicated indirectly by a decrease in the dry weight per unit area of the frond and by a decreasing carbohydrate content (particularly mannitol but also to a lesser extent laminarin) of both the mature and the new frond tissue (see chapter 7). Since reserves would appear to be so important in supporting new frond growth, removal of most of the carbohydrate reserves in October would be expected to reduce the quantitative response but not remove the qualitative response - new frond growth would still be initiated at the same time but the growth rates expressed would be very much lower. This was supported by evidence from L. saccharina but excision of L. digitata old frond did not significantly reduce new frond growth rates, possibly because of the greater width of storage area in the broader frond. Perez (1967; 1968; 1971) reported that excision of the lamina of young L. digitata produced variable results on subsequent spring growth depending on the age of the plant and the time of the excision (whether September or November). Perhaps excision in September/October allows sufficient time for photosynthesis to replace some of the carbohydrate reserves removed and growth, which is still fairly rapid

at this time will increase the area for both photosynthesis and nutrient uptake. In addition, removal of much of the mature tissue reduces the respiratory demand as light decreases. If, however, the excision is made in November, growth rates are at a minimum and spring growth begins with a very significantly reduced area (reduced even compared to September excised plants) the small area is insufficient for adequate nutrient uptake and photosynthesis (when light is sufficient to allow a photosynthetic surplus) and little area of reserve to draw upon, hence subsequent spring growth is significantly reduced. The relationship between frond length and growth rate is not clear in L. digitata, however and further work is required on this aspect of new frond growth.

Stored reserves, which appear so important to spring growth in L. saccharina and also probably L. digitata are only of use to the plant if sufficient of the carbohydrate-rich frond tissue remains intact by January/February to support new frond growth demands. Measurements of the rate of frond erosion suggest that a large proportion (over 50%) of the frond tissue present in October remains available to the alga in January. These figures cannot be taken as absolute values because of the wide variation in the rate of frond erosion, depending on the position of the plants on the shore and the prevailing weather conditions, but they do provide useful indications of the degree of tissue retention by L. saccharina and L. digitata at St. Andrews. This is similar to the figure quoted for

L. longicruris (57% of the November frond tissue was still present by January; Chapman & Craigie, 1978) but these authors concluded that reserve carbohydrates did not play a significant role in supporting winter growth in this species since both laminarin and mannitol content had declined significantly by November and 43% of this low-carbohydrate content tissue had been lost by January. However, in L. saccharina and L. digitata the mannitol content of the frond is still relatively high by December/January (Chapter 7) and may, therefore, be used extensively as an energy source.

New frond growth in L. digitata and L. saccharina appears to utilise reserves accumulated the previous summer, and despite continual attrition of the distal tissue a large proportion of the mature tissue is retained. There is ample evidence in the literature to show that translocation of mannitol (one of the major translocatory products) occurs from the distal to the young tissue at this time (see review: Schmitz & Lobban, 1976). However, there is controversy as to the actual contribution of the translocated materials to new frond growth (see review: Kain, 1979) but most of the evidence suggests that retention of the old frond (or a large proportion of it) is important for normal spring growth.

Retaining the old frond during the winter subjects the holdfast and stipe to severe mechanical stresses, particularly during turbulent weather and there is a serious

risk of the whole plant being pulled away from the substrate. There must, therefore, be a great strategic advantage outweighing the dangers in retaining the old frond, compared with shedding the tissue in the autumn, as in deciduous higher plants. The role of the old frond as a storage organ for carbohydrates accumulated in the previous summer has been elucidated above but the old frond appears to have several additional functions.

Results presented here indicate that the old frond is important in providing an increased area for nitrate and phosphate uptake and basal translocation then brings these nutrients to the actively growing new frond. This is shown by a higher nitrogen and phosphate content of the new frond when the old frond remains attached than when the new frond is isolated and hence relying on its own uptake capacity. There is evidence (using radiotracers) in the literature that both N compounds (predominantly as amino acids: Schmitz et al, 1972) and P compounds (Floc'h & Penot, 1971; 1972; 1974; 1976) are translocated basally in Laminaria. The new fronds (-old frond) grown in a range of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations showed that the young tissue apparently has a limited capacity for nitrate uptake, since the tissue could not take advantage of the high external concentrations supplied, but that phosphate was taken up in proportion to the supply concentration. This suggests that the ion uptake capacity and possibly also the velocity of uptake by growing tissue

is very less than that of mature non-growing tissue. The cell resources are diverted from uptake to growth. This phenomenon occurs in other plant tissues eg. Artichoke (Steward & Millar, 1954). The implication for Laminaria is that the old frond is, therefore, essential to new frond growth by increasing the area for new frond growth. Initially, the new frond may be dependent to a large extent on mineral supply from the old frond as the cells divert resources to growth, but as the area increases and the cells move beyond the basal meristem, dependence on the old frond probably lessens as more of the expanding and fully grown cells are diverted from growth to mineral uptake.

In addition to acting as a store for carbohydrate, the old frond may also act as a reserve for both N and P. When the old frond is attached, rapid growth rates of the new frond are maintained, even in depleted seawater, by utilising N or P reserves of the old frond. The old frond provides a pool of N and P compounds which may be drawn upon, as necessary, and although the isolated new frond is able to maintain adequate growth rates, it is utilising its own reserves, presumably translocated from the old frond previously, because of the active meristem's own limited ability for nutrient uptake. Excision of the lamina in October, in situ, removes carbohydrate reserves, but also reduces the surface area for nutrient uptake as seawater N and P increase during the autumn and winter. It

is unlikely that this would be significant in reducing subsequent spring growth rates of L. saccharina as the external concentrations of N and P are probably saturating growth and uptake requirements (see subsequent chapters). However, during the early stages of new frond growth in both L. saccharina and L. digitata the new frond is probably relying almost entirely on the old frond for uptake, storage and translocation of N and P. In the absence of the old frond, either experimentally or by natural attrition, and despite high external nutrient concentrations, the new frond may be severely nutrient-limited.

The fourth possible function of the old frond in increasing the area for photosynthesis requires more detailed information on in situ irradiances before the importance of this role can be ascertained. Although continuous underwater light measurements have been made (Luning & Dring, 1979) the actual amount of light received by these lower intertidal algae varies with position on the shore and hence the length of time uncovered and the ability of Laminaria to photosynthesize when emersed. It is probable that for long periods during the winter, irradiance is below the light compensation point for photosynthesis, but an increased surface area would be an advantage to the new frond when irradiance was sufficient for a photosynthetic surplus. However, the relationship between growth and photosynthetic area may not be a simple function of total photosynthetic area (this is the case in L. longicruris

(Chapman & Craigie, 1978)) and although growth rate is a linear function of frond length (up to 50 cm frond length) in L. saccharina (Luning et al, 1973) the basal meristem is partly dependent on its own assimilation; the basal 12% of the frond length assimilates 30% of the carbon required for growth, 70% is translocated from the mature frond tissue (Luning et al, loc cit). It is, therefore, difficult to distinguish the effects of translocation from those of photosynthetic production of the old frond.

The old frond, therefore, appears to have 4 supportive functions to the new frond in January/February. It acts as a store for carbohydrates accumulated the previous summer and it is very important in increasing the area for mineral uptake particularly since the new frond itself appears to have only a limited uptake ability for nitrate. The old frond acts as a store for the N and P taken up and fourthly, it may be important in increasing the photosynthetic area when irradiance is adequate for a photosynthetic surplus. In addition the old frond may supply vitamins and growth-promoting substances to the new frond which act in combination with the roles already described. The supply of carbohydrates and nutrients (N and P) suggest that the developing new frond (at least in the early stages) may be largely independent of chemical raw materials and light, and is therefore, almost completely reliant on the old frond.

Laminaria sporophytes may undergo "senescence" or

a change in cell fate with age. Such a change in cell fate can be looked at from 3 levels from both a macro- (relating to the whole plant) and at a micro-aspect (the changing fate of individual cells).

- i Senescence with plant age: the effect of age on whole plant growth.
- ii Senescence with season: concerned only with frond age and changes over the year in the ability of the frond to respond to changing environmental conditions.
- iii Senescence with distance along the frond: aging of individual cells from their production in the transition zone to displacement distally with the loss of meristematic activity.

Senescence with plant age. Although the results are inconclusive, there is an indication that increasing plant age results in a decreased frond growth potential or a decline in the maximum linear growth rates (and hence, decreased total area) achieved by fronds produced by the same plant in successive years. A smaller frond area produced with increasing age would necessarily result in a smaller frond produced the following year, because of the reduction in available reserves for new frond growth (for L. saccharina but not necessarily in L. digitata indicated by the October excision experiments). The majority of plants are probably pulled off the substrate before this self-perpetuating system produces frond areas insufficient to support subsequent new frond growth. Previous work

has concentrated on stipe and lamina weight plots against age, mainly in L. hyperborea (Kain, 1963; 1976 ; Jupp & Drew, 1974). However, stipe weight (and length) varies significantly between populations (between closed and open canopy plants) and lamina weight is not a good indication of area and hence growth because of variation during the year of fresh weight/dry weight ratios. In addition, area itself is difficult to measure as continual attrition removes distal tissue.

Senescence with season. This is discussed more thoroughly in subsequent chapters, but the declining growth rates during the year, particularly in late summer when light and temperature are high and nutrients are increasing, may indicate that the frond has lost its springtime ability for rapid and extensive cell division and cell expansion. In addition, the qualitative change between cell response to increasing photoperiod between November and December suggests senescence may be a major consideration in the seasonal growth pattern of Laminaria.

Senescence with distance along the frond. Almost all of the lamina growth occurs in the basal 10 cm of the frond of L. saccharina and L. digitata, the capacity for cell division and enlargement is lost to a great extent as the cells are displaced distally. During this change in cell fate resources may be diverted from growth to mineral uptake (see previously). Only under certain circumstances will cell division/enlargement be "switched on" again in the mature frond tissue, eg. damage caused by

crazing or attrition may result in the wounds healing by the development of flaps of tissue over the damaged surfaces (and such flaps also develop around the cut edges when holes are punched for growth measurements).

Senescence at all 3 levels may be important in influencing the seasonal pattern of growth in Laminaria.

To summarise: the seasonal growth pattern of L. saccharina and L. digitata is demonstrated, but temperature and light (photoperiod and irradiance) alone are unable to adequately explain the increasing growth rates in January, the decrease in growth after a maximum in May or the transient increase in growth rates in September/October. In situ, light (photoperiod and irradiance) and temperature in the summer are not inhibitory to growth of L. saccharina or L. digitata and cannot account for the growth rate decline in May. The role of nutrients at this time is investigated in subsequent chapters.

Temperature and light appear limiting to growth of L. saccharina and L. digitata during November-January but new frond growth appears to be "triggered" by increasing daylength after the shortest day, by initiating rapid cell division. New frond growth can be initiated in the laboratory in December by increasing the photoperiod. Growth once started relies extensively on stored carbohydrate reserves accumulated in the previous summer, and despite continual attrition more than 50% of the tissue present in October remains attached by the following January. The presence of the old frond is necessary for rapid new frond

growth since it acts as a reserve for carbohydrate, N-compounds and P-compounds, increases the photosynthetic area and increases the surface area for nutrient uptake. The latter function might be important as the actively growing cells of the new frond appear to have only a limited nitrate-uptake capability.

A change in cell fate or "senescence" of the frond tissue with plant age, season and with distance along the frond may also be important in influencing the seasonal pattern of linear frond growth in L. saccharina and L. digitata.

CHAPTER 4

NITROGEN AND GROWTH

INTRODUCTION

It is widely accepted that nitrogen is important in limiting marine phytoplankton productivity; the close relationship between the summer decline in phytoplankton biomass and the depletion of inorganic nutrients (particularly N and P) is well documented (see Harvey, 1926; Ryther & Dunstan, 1971; Horwood, 1982; for details). The effect of inorganic nutrients on the seasonal growth of marine macroalgae is poorly understood.

Black & Dewar (1949) first attempted to correlate seasonal variations in the chemical composition of Laminaria spp. with changes in seawater nutrient levels. Indirect estimates for the inorganic nitrogen content of L. saccharina showed that it was elevated in winter and low during the summer. Measuring the inorganic-N content directly, Channing & Young (1953) and Larsen & Jensen (1957) showed a similar seasonal fluctuation. Such changes were not investigated in relation to algal growth rates but the low summer tissue-nitrate content suggested that seawater N concentrations might limit growth at this time. There appears to be a close correlation between internal nitrate and seawater nitrate concentration as the decline of winter accumulated nitrate reserves of L. longicruris followed the disappearance of external nitrate after a lag period of up to 2 months (Chapman & Craigie, 1977). L. solidungula

(Chapman & Lindley, 1930) and Nereocystis luetkeana (Whyte & Englar, 1975) also show a similar seasonal variation of internal nitrate. The organic nitrogen reserves of the Laminariales also showed a marked seasonal variation. Proteins are at a maximum during the winter in the frond of L. digitata, L. saccharina and L. hyperborea, dropping rapidly to a minimum from July-September (Black, 1948; Black & Dewar, 1949; Haug & Jensen, 1954; Jensen & Haug, 1956). Similar seasonal fluctuations occur in other brown algae; Fucus virosoides, Sargassum vulgare (Munda, 1962) and Alaria esculenta (Haug & Jensen, 1954).

The seasonal variation in the pattern of linear growth rates of Laminaria spp have been well known since the work of Parke (1948); growth rates show a dramatic increase in late winter reaching a peak in May followed by a rapid decline in linear growth rates during the summer. This capacity for winter growth has attracted attention because light and temperature are low at this time in temperate latitudes. In L. longicruris rapid growth starts soon after ambient nitrate levels rise dramatically and as nitrate falls in the Spring, growth declines (Chapman & Craigie, 1977). Nutrient limitation during the summer has been demonstrated and the spring decline in growth rate of L. longicruris was prevented by nitrate-enrichment in situ and growth rates in midsummer approximated those of control plants in early spring. Buggeln (1974) found both nitrate and phosphate to be limiting growth of Alaria esculenta

during the summer. Such investigations have not previously been made on British Laminaria species. This study aims to contribute towards this aspect in an attempt to determine the importance of nitrogen as a growth controlling factor of L. saccharina and L. digitata.

While extensive research has been carried out on the nutrient physiology of phytoplankton, relatively little is known about the nutrient requirements of benthic algae, particularly their possible N assimilation preferences (Bird, 1976). However, recent research has studied uptake of nitrogen sources by a number of macroalgae including Macrocystis pyrifera (Haines & Wheeler, 1977), Fucus spiralis (Topinka, 1978), L. longicruris (Harlin & Craigie, 1978), Enteromorpha spp (Harlin, 1978), Neogardhiella baileyi (D'Elia & DeBoer, 1978) and growth on a range of nitrogen concentrations has been demonstrated for L. saccharina (Chapman et al. 1978), Fucus spiralis (Topinka & Robbins, 1976) and Codium fragile (Hanisak, 1979a). Preference for one nitrogen form over another has been investigated (Fries, 1963; Iwasaki, 1967; Nasr, Bekheet and Ibrahim, 1968; Rao & Mehta, 1973) as also has the simultaneous assimilation of nitrate and ammonium (Neish & Fox, 1971; Neish & Shacklock, 1971; Prince, 1974).

Growth on different nitrogen sources, kinetics of nitrate uptake and enrichment experiments were carried out in the laboratory to investigate possible nitrogen source preferences in L. saccharina and L. digitata. These

Fig. 4 i. Seawater nitrate concentration during 1979-1981 at Kingsbarns and Fifeness.

Fig. 4 ii. Seawater nitrate concentration during 1979-1981 at St. Andrews and St. Andrews Sewer.

Fig. 4 i.

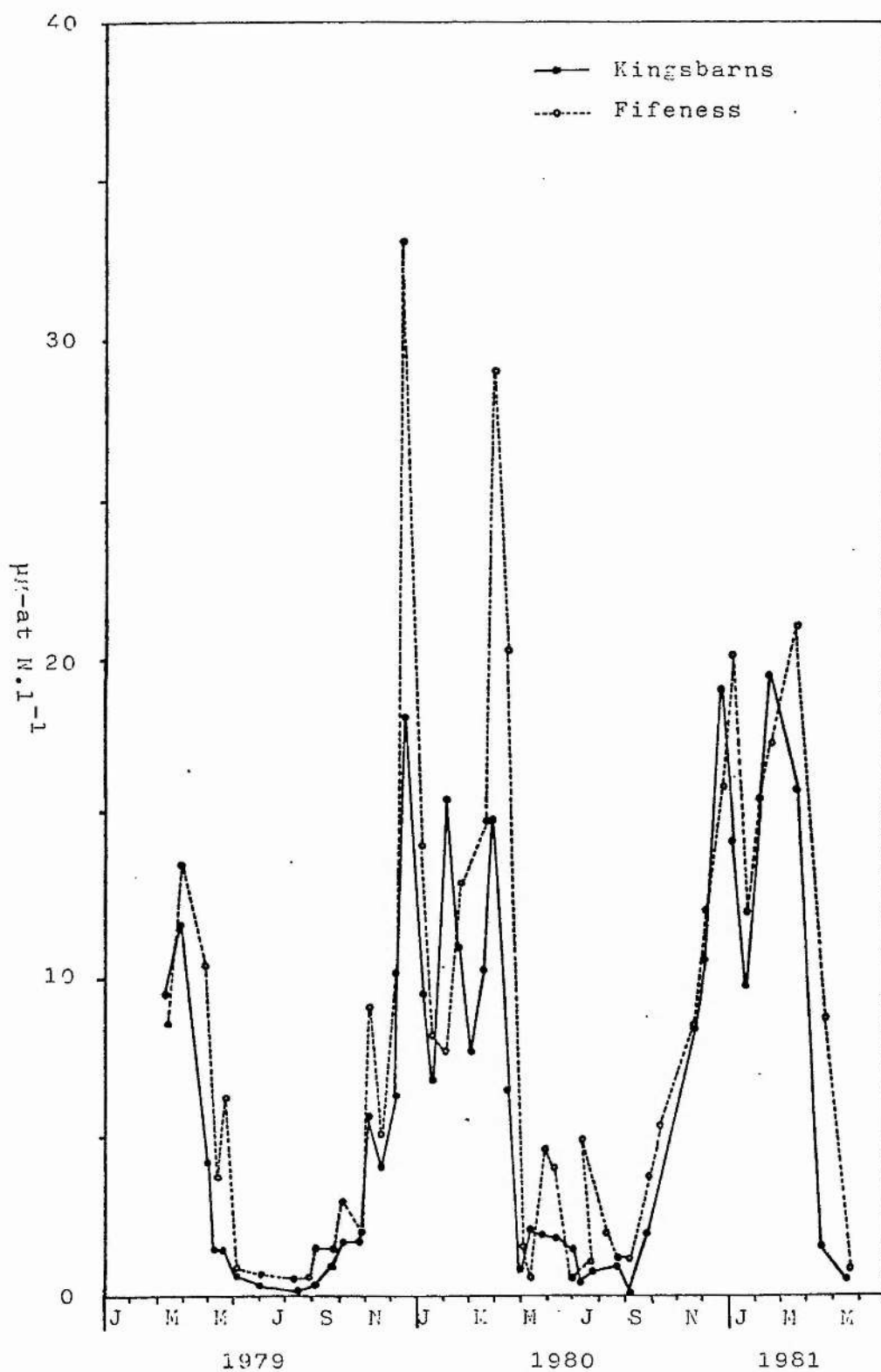


Fig. 4 ii.

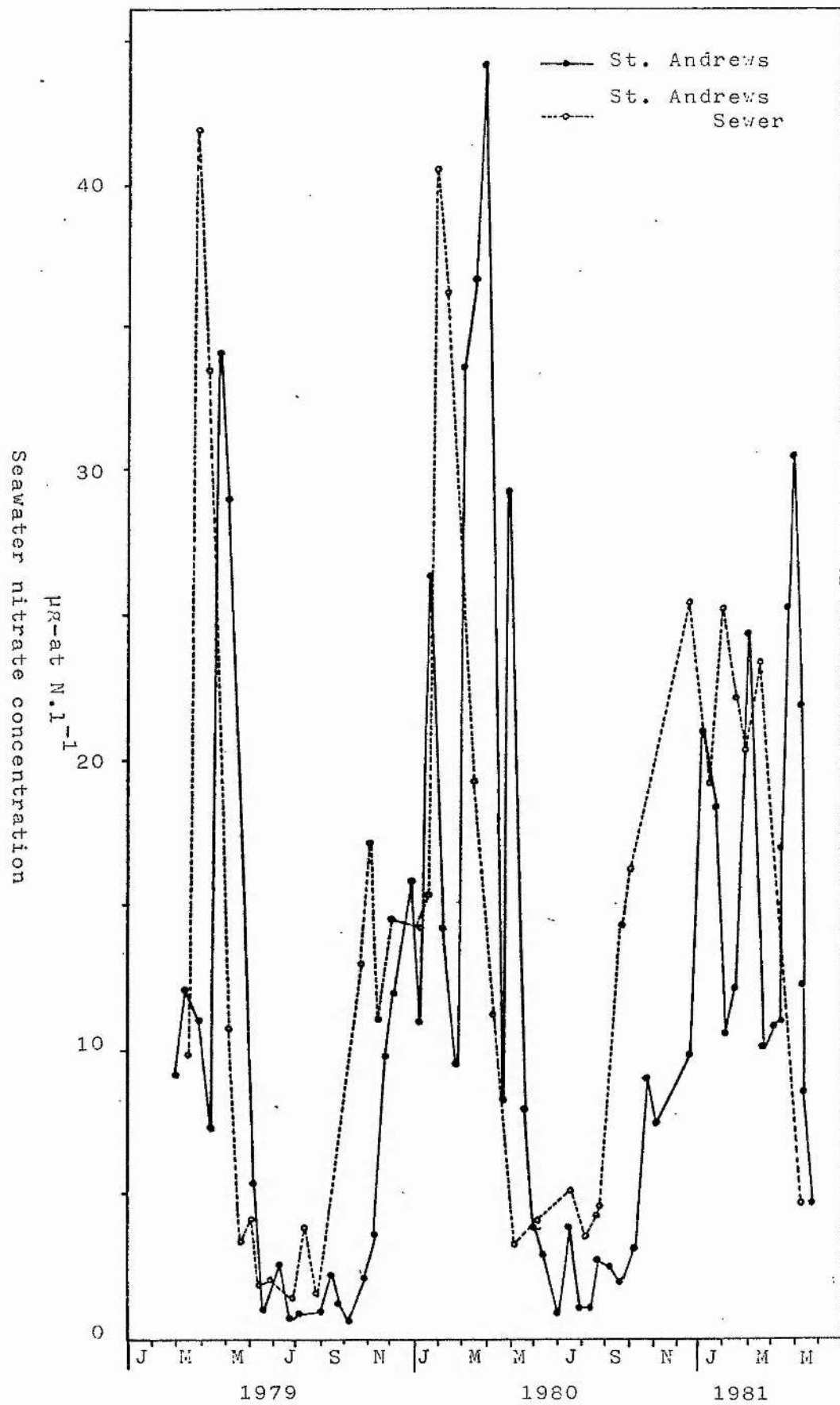
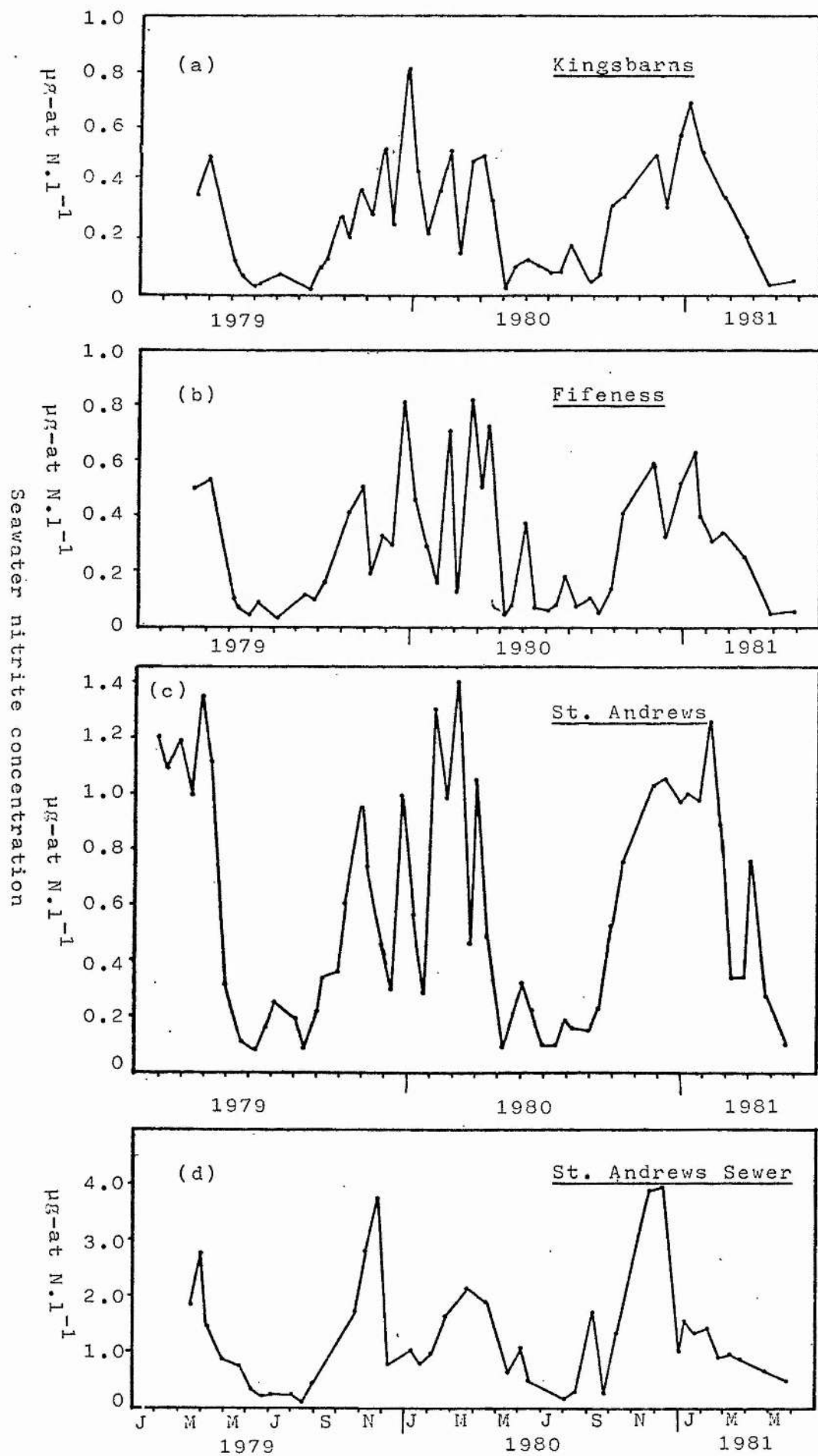


Fig. 4 iii. Seawater nitrite concentration 1979-1981.



results are discussed in relation to the chemical composition of the algae and the seasonal fluctuation of nitrogen in the seawater to estimate the importance of nitrogen in affecting or controlling seasonal growth rates of Laminaria. The possibility of N limitation of summer growth is discussed.

RESULTS

i FLUCTUATION IN SEAWATER N

The levels of nitrate, nitrite and ammonium were monitored at the 4 sampling sites from 1979-1981. Features of note to be discussed below are:

- a Seasonal variation in NO_3 , NO_2 and NH_4
- b Site Variation
- c Relative proportions of $\text{NO}_3:\text{NO}_2:\text{NH}_4$
- a Seasonal Variation

Nitrate (Figs 4 i+ii) and nitrite (Fig. 4 iii) show a similar seasonal fluctuation in the sea but concentrations of nitrite are more than an order of magnitude lower than nitrate. The rapid decline in seawater nitrate and nitrite in April occurs as phytoplankton density peaks in the Spring. The low external concentrations are maintained during the summer as vertical mixing is restricted and the nitrate and nitrite becomes locked up in algae, zooplankton and the sediments. It is not until the autumn (September) that nitrate and nitrite concentrations increase with increasing turbulence.

Ammonium (Figs 4 iv+v) varies from this typical seasonal

Fig. 4 v. (Facing page). Seawater ammonium concentration during 1979-1981 at St. Andrews and St. Andrews Sewer.

Fig. 4 iv. Seawater ammonium concentration during 1979-1981 at Kingsbarns and Fifeness.

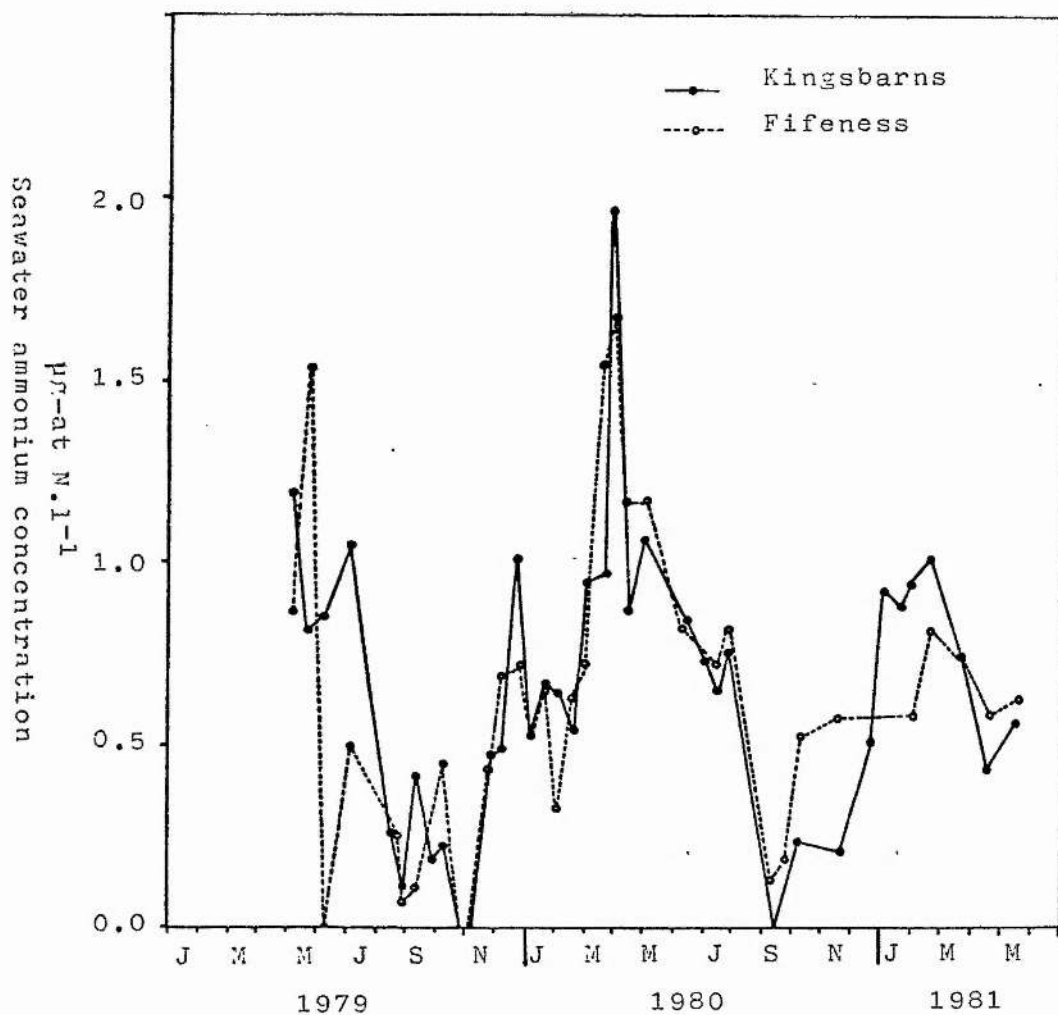
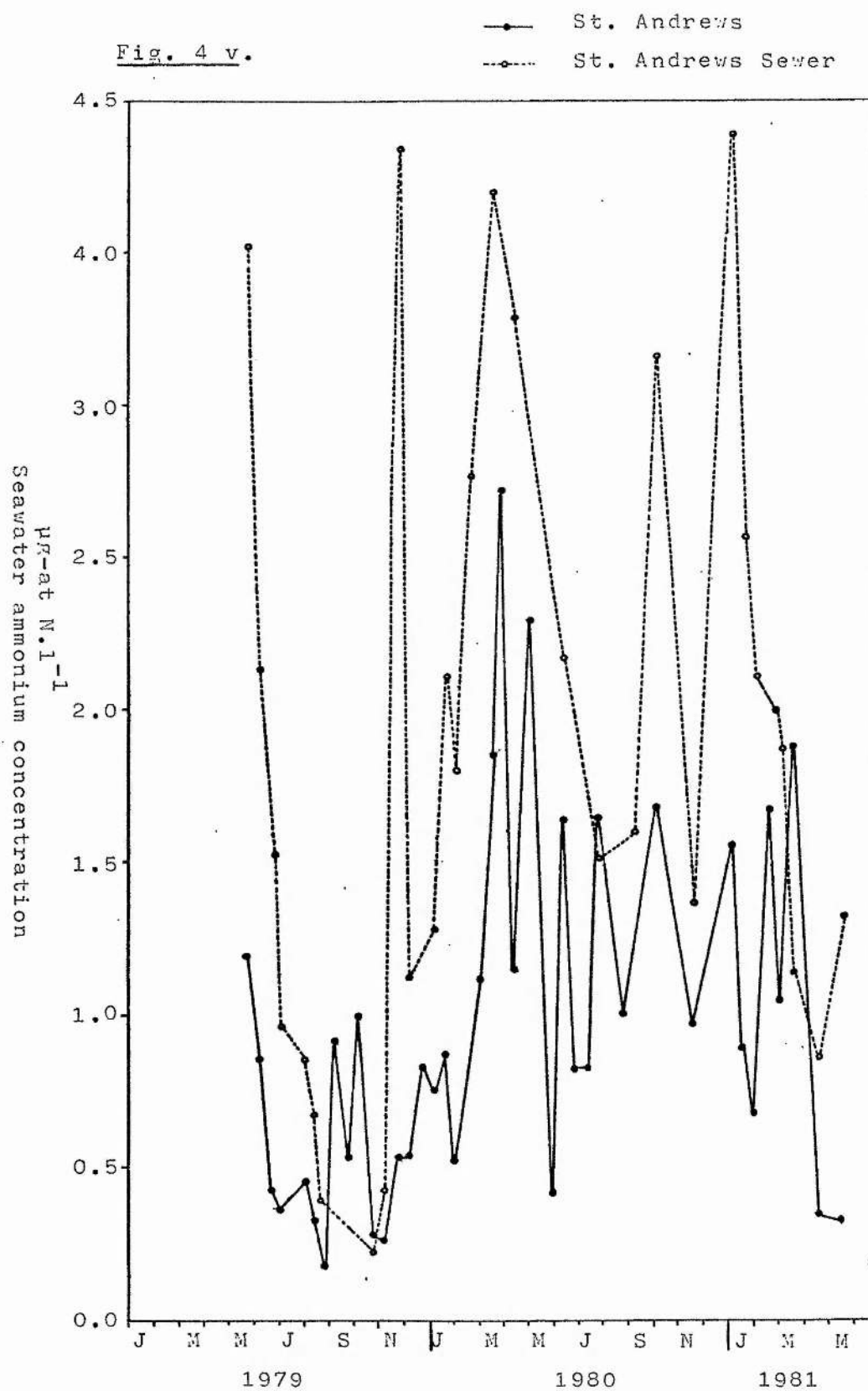


Fig. 4 v.



pattern and there are considerable fluctuations between the concentrations recorded on successive sampling dates. Results for January-April 1979 were considered technically unreliable and have not been included. $\text{NH}_4\text{-N}$ is the excretory product of most marine organisms, it is produced during bacterial decomposition and, in addition, input from rivers, run-off and pollution influence levels found in the sea. The higher summer levels might result from an increased $\text{NH}_4\text{-N}$ production as zooplankton density peaks and domestic sewage input increases during the summer holiday season.

b Site Variation

Nitrate. The mean summer and winter concentrations at the 4 sites are shown in Table 4 i.

Table 4 i. The mean summer (1980) and winter (1980/81) concentrations of nitrate at the 4 sampling sites. Mean \pm SE.

Site	Summer $\mu\text{g-at N.l}^{-1}$	Winter $\mu\text{g-at N.l}^{-1}$
Kingsbarns	0.72 ± 0.19	13.13 ± 1.79
Fifeness	0.86 ± 0.17	14.27 ± 1.74
St. Andrews	1.22 ± 0.26	15.87 ± 2.07
Sewer	2.65 ± 0.98	20.22 ± 1.39

Kingsbarns, Fifeness and St. Andrews are not statistically different with regard to the seawater nitrate con-

centration. However, nitrate at St. Andrews Sewer is significantly higher than St. Andrews ($P < 0.02$; NS), Kingsbarns ($P < 0.001$; $P < 0.01$) and Fifeness ($P < 0.01$; $P < 0.02$) during the summer and winter respectively. The higher nitrate concentration at Sewer is probably caused by domestic sewage input.

Nitrite. Fifeness and Kingsbarns exhibit similar summer and winter mean concentrations (Table 4 ii). St. Andrews is significantly higher than these 2 sites in the summer ($P < 0.02$) and winter ($P < 0.001$), but, as with nitrate above, Sewer is significantly higher than St. Andrews ($P < 0.05$) and at Kingsbarns and Fifeness ($P < 0.01$) in the summer and $P < 0.05$ and $P < 0.01$ at the sites respectively during the winter. Once again the influence of the domestic sewage input is obvious.

Table 4 ii. The mean summer (1980) and winter (1980/81) concentrations of nitrite at the 4 sampling sites. Mean \pm SE

Site	Summer	Winter
	$\mu\text{g-at N.l}^{-1}$	$\mu\text{g-at N.l}^{-1}$
Kingsbarns	0.089 ± 0.014	0.427 ± 0.051
Fifeness	0.112 ± 0.028	0.418 ± 0.044
St. Andrews	0.205 ± 0.040	0.815 ± 0.087
Sewer	0.656 ± 0.211	1.707 ± 0.372

Ammonium. Because there is no spring and summer decline

in ammonium in contrast to nitrate and nitrite, a single figure for the mean annual concentration is calculated and compared between sites. This mean annual value for 1980 is similar at Kingsbarns and Fifeness (0.76 ± 0.10 and $0.70 \pm 0.10 \mu\text{g-at N.l}^{-1}$ respectively) while at St. Andrews is significantly higher, $1.22 \pm 0.16 \mu\text{g-at N.l}^{-1}$ ($P < 0.05$). The ammonium concentration is significantly higher at the Sewer than St. Andrews ($P < 0.002$) and Kingsbarns and Fifeness ($P < 0.001$) with a mean annual $\text{NH}_4\text{-N}$ concentration of $2.24 \pm 0.26 \mu\text{g-at N.l}^{-1}$. The raised NH_4 concentration probably results from domestic sewage input and because of the importance of sewage input on the concentrations of all 3 nitrogen forms, the sewage treatment process employed at St. Andrews is outlined below.

At St. Andrews, the sewage effluent is discharged just below extreme low water of spring tides (ELWS) continuously over 24 hours. The raw sewage undergoes primary treatment (settlement only, not accompanied by biological degradation) and the final discharge has a B.O.D. (Biochemical Oxygen Demand) of about 150 ppm and 100 ppm suspended solids. The discharge of the effluent depends on the frequency of pumping to the primary tanks which, in turn, varies with the weather conditions. The inflow to the sewage works is monitored; the amount and rate of discharge is not but can be estimated to be about 58,000 gals/day (about 40 gals/min). Although there is apparently chemical checks on the water content leaving the outlet approximately twice a year, no such analyses appeared to

have been made during the period under study and hence, no official records of the chemical composition (in particular for N and P) of this effluent were obtained for comparative purposes. The effluent is rapidly dispersed and diluted from the outlet as indicated by the difference in N composition of the seawater collected at the outlet (St. Andrews Sewer site) and at approximately 120 m east at the St. Andrews site. However, the effluent results in higher nitrate, nitrite and ammonium concentrations at St. Andrews than at the 2 relatively unpolluted sites (Kingsbarns and Fifeness).

c Relative proportions of nitrate:nitrite:ammonium

During the summer nitrate and ammonium occur in similar proportions relative to nitrite at the 4 sites (Table 4 iii).

Table 4 iii. Relative proportions of nitrate, nitrite and ammonium in the summer (1980) and winter (1980/81) at the 4 sampling sites.

Site	Summer			Winter		
	NO ₃	: NO ₂	: NH ₄	NO ₃	: NO ₂	: NH ₄
St. Andrews	5.95	: 1	: 5.95	18.65	: 1	: 1.43
Sewer	4.04	: 1	: 3.42	11.85	: 1	: 1.31
Kingsbarns	8.09	: 1	: 8.54	30.75	: 1	: 1.78
Fifeness	7.68	: 1	: 6.25	34.14	: 1	: 1.67

During the winter nitrate comprises the greatest

proportion of seawater N; at Kingsbarns and Fifeness this is about 32 times the concentration of nitrite. At Sewer and St. Andrews the actual concentrations of N are higher than elsewhere, but all 3 nitrogen fractions are raised and there is less nitrate relative to nitrite and ammonium than at Kingsbarns and Fifeness.

The relative proportions of the 3 nitrogen sources may be important if the algae exhibit any particular preference for growth or uptake of one form over another or if uptake of one N source is suppressed in the presence of the other(s).

ii INTERNAL NITROGEN

Using the method of Chapman & Craigie (1977) the nitrogen content of the frond was separated into protein, non-protein (low molecular weight organic compounds, amines, amides, peptides etc) and inorganic-N (nitrate). The results are considered under the following headings:

- a Seasonal Variation
- b Variation between sites
- c Differences in seasonal variation between meristematic and mature tissue
- d Variation between species
- e Relative proportions of Protein, Non-Protein and inorganic-N

a Seasonal Variation

L. saccharina. In the mature frond tissue, total N per unit dry weight of tissue increases to a maximum in

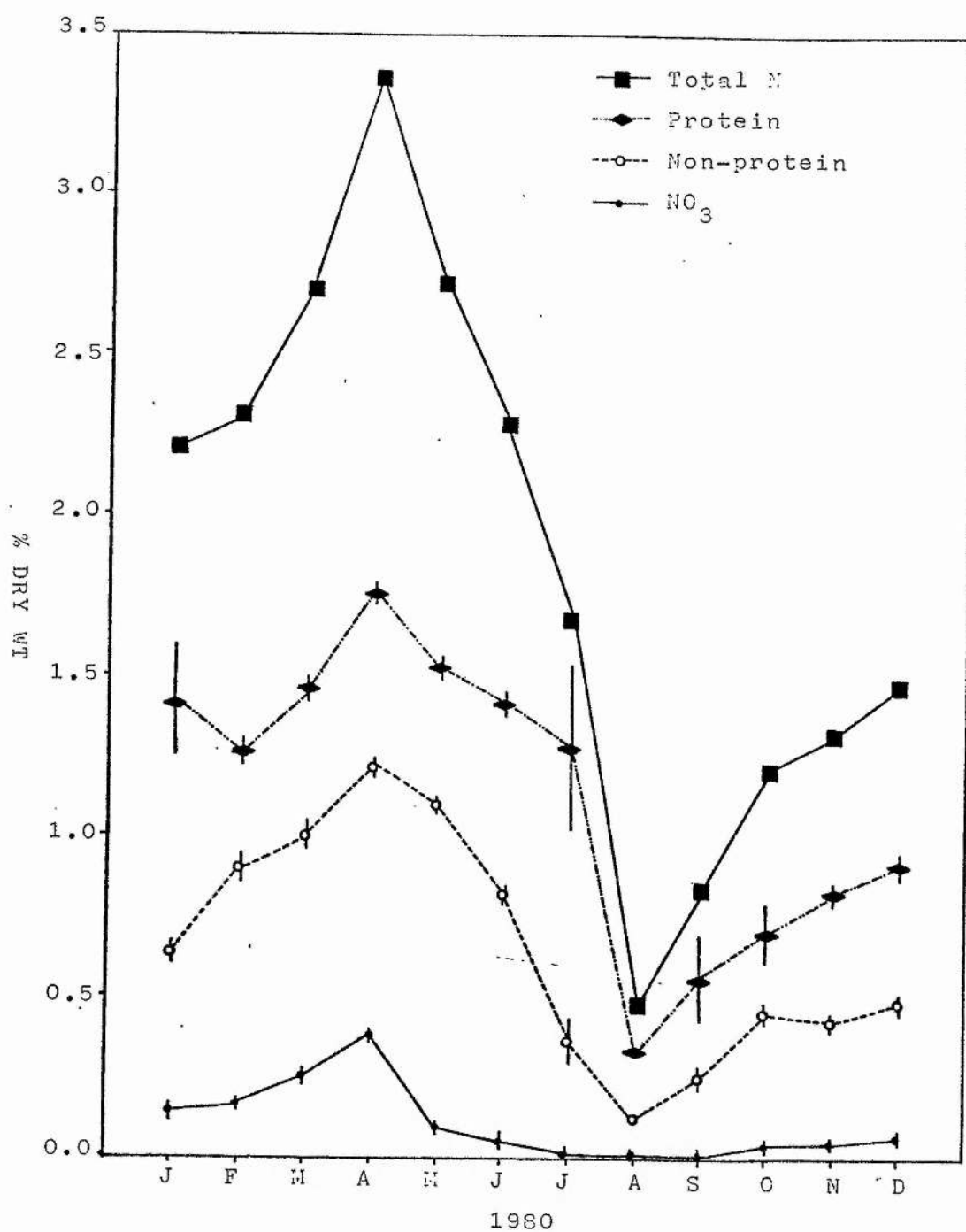


Fig. 4 vi. Internal N content (% dry wt) of the mature frond tissue of *L. saccharina* at St. Andrews during 1980. (Mean \pm SE with 3 replicates/treatment)

Fig. 4 vii. Internal N content (% dry wt) of the meristem of *L. saccharina* at St. Andrews during 1980. (Mean \pm SE with 3 replicates/treatment).

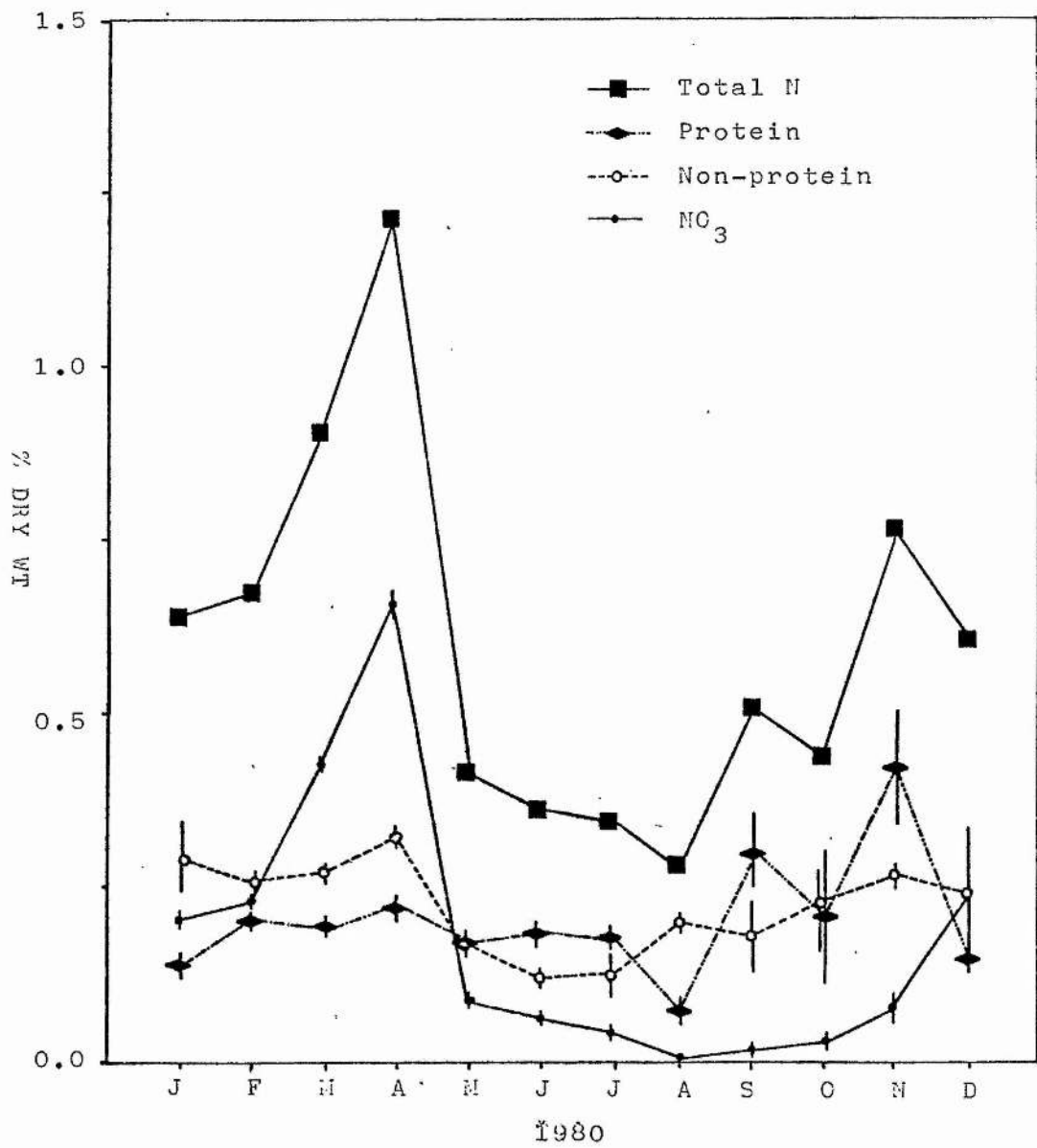


Fig. 4 viii. Internal N content (% dry wt) of the mature frond tissue of *L. digitata* at St. Andrews during 1980. (3 replicates/treatment. Mean \pm SE)

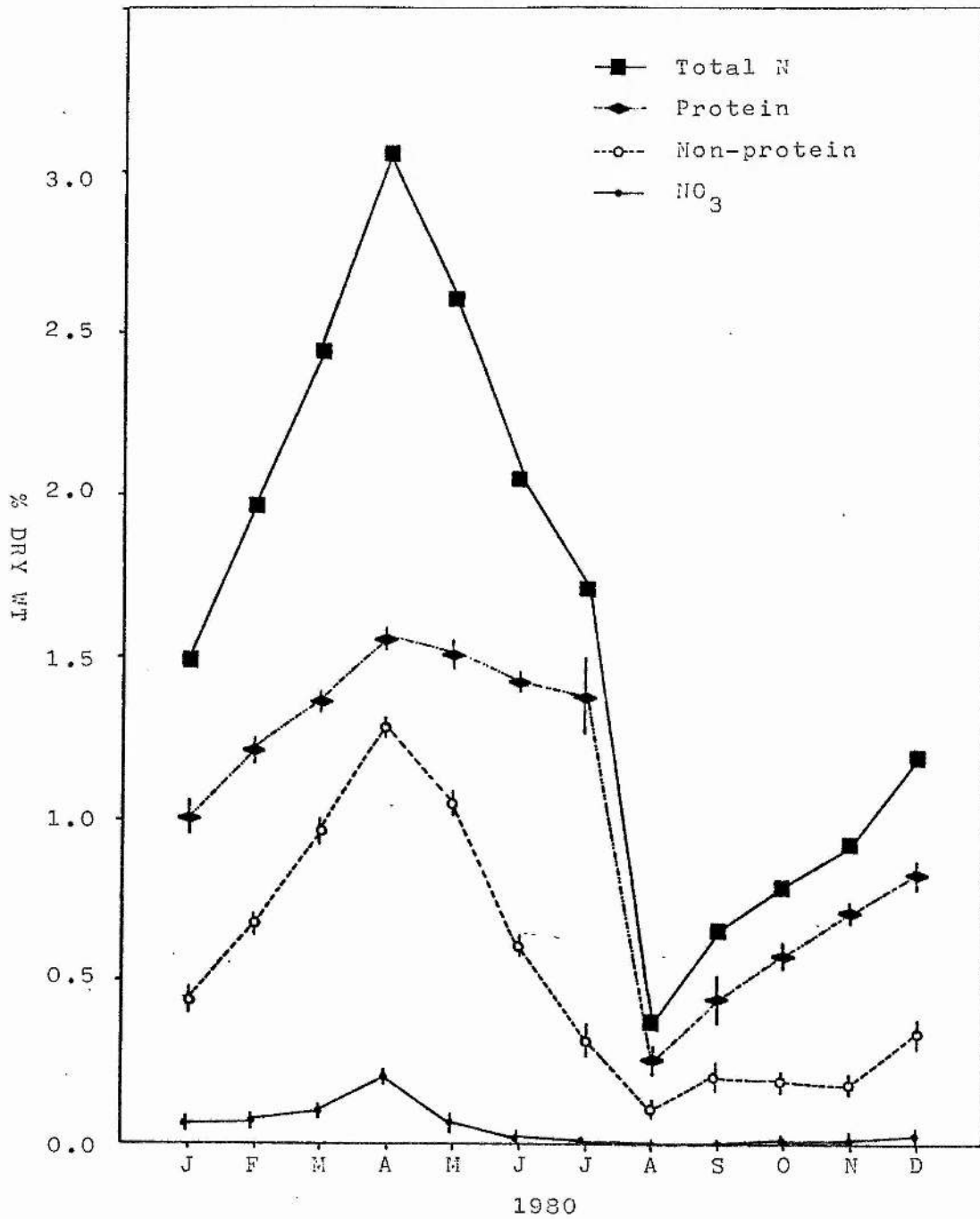


Fig. 4 ix. Internal N content (% dry wt) of the meristem of *L. digitata* at St. Andrews during 1980. (Mean \pm SE with 3 replicates/treatment)

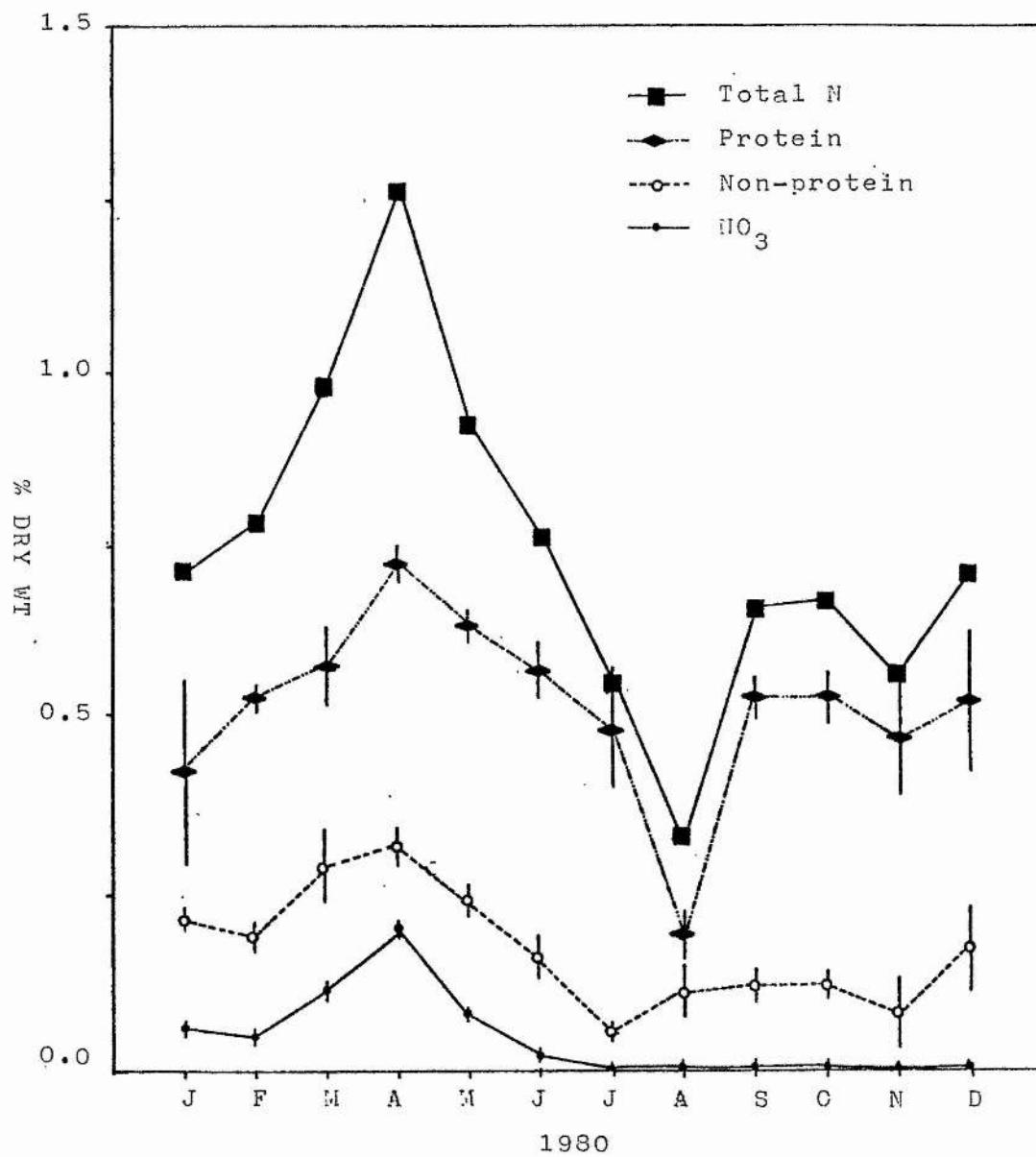


Table 4 iv a. Internal N content (% dry wt) of mature frond tissue of L. saccharina from the 4 sampling sites.

1980	Site	Total N	Protein	Non-protein	Inorganic-N
Jan	KB	0.74	-	-	-
	FN	0.68	-	-	-
	St. A	2.21	1.43	0.64	0.15
	SEW	2.50	-	-	-
Apr	KB	1.30	-	-	-
	FN	1.18	-	-	-
	St. A	3.37	1.76	1.22	0.39
	SEW	3.51	-	-	-
July	KB	1.17	0.99	0.18	0.00
	FN	0.91	0.87	0.04	0.00
	St. A	1.68	1.28	0.38	0.01
	SEW	1.73	1.41	0.31	0.00
Sept	KB	0.63	0.50	0.13	0.01
	FN	1.37	1.20	0.14	0.03
	St. A	0.84	0.57	0.26	0.01
	SEW	0.78	0.50	0.27	0.01
Oct	KB	0.66	0.46	0.19	0.01
	FN	0.67	0.45	0.21	0.01
	St. A	1.22	0.71	0.46	0.04
	SEW	1.03	0.89	0.13	0.01

KB = Kingsbarns
St. A = St. Andrews

FN = Fifeness
SEW = Sewer

April before dropping sharply to an August minimum, and then gradually increasing again during the autumn and winter (St. Andrews data, Fig. 4 vi). After the April maximum inorganic-N drops very steeply as too does non-protein N whereas protein declines only gradually until July.

In the meristem (St. Andrews data, Fig. 4 vii) the pattern of total-N is similar to the mature tissue with non-protein and inorganic-N dropping sharply in May to low summer levels.

L. digitata. The seasonal pattern of internal N in the mature frond tissue (Fig. 4 viii) and in the meristem (Fig. 4 ix) is similar to that shown by L. saccharina mature tissue at St. Andrews.

b Variation between sites

L. saccharina. The seasonal variation of internal N and the actual N content is similar at Kingsbarns and Fifeness with an April maximum in the meristem of 1.39% and 1.40% dry weight respectively (Fig. 4 x(a-b)). The meristem tissue N content of St. Andrews plants was not significantly different from this, while Sewer (Fig. 4 x(c)) plants were significantly higher. In the mature tissue, St. Andrews and Sewer plants had more than double the relative N content of plants from the other 2 sites (Table 4 iv a).

L. digitata. From January to July, the tissue N (total) content at St. Andrews and Sewer is considerably higher

Fig. 4 x. (See facing page for legend)

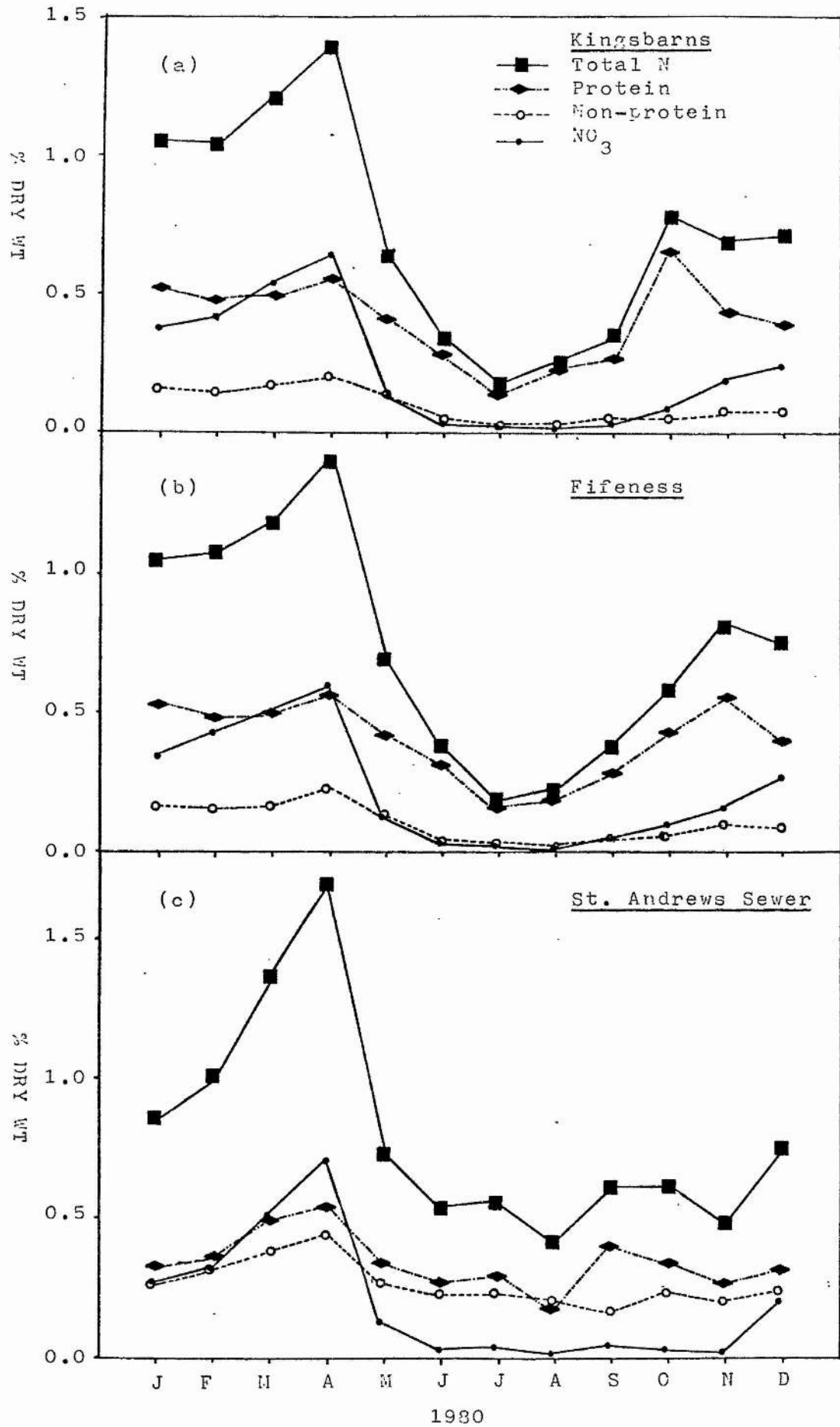


Fig. 4 x. (Facing page) Internal N content of the meristem of *L. digitata* during 1980 at (a) Kingsbarns, (b) Fifeness and (c) St. Andrews Sewer.

Fig. 4 xi. Internal N content of the meristem of *L. digitata* during 1980 at (a) Kingsbarns, (b) Fifeness and (c) St. Andrews Sewer.

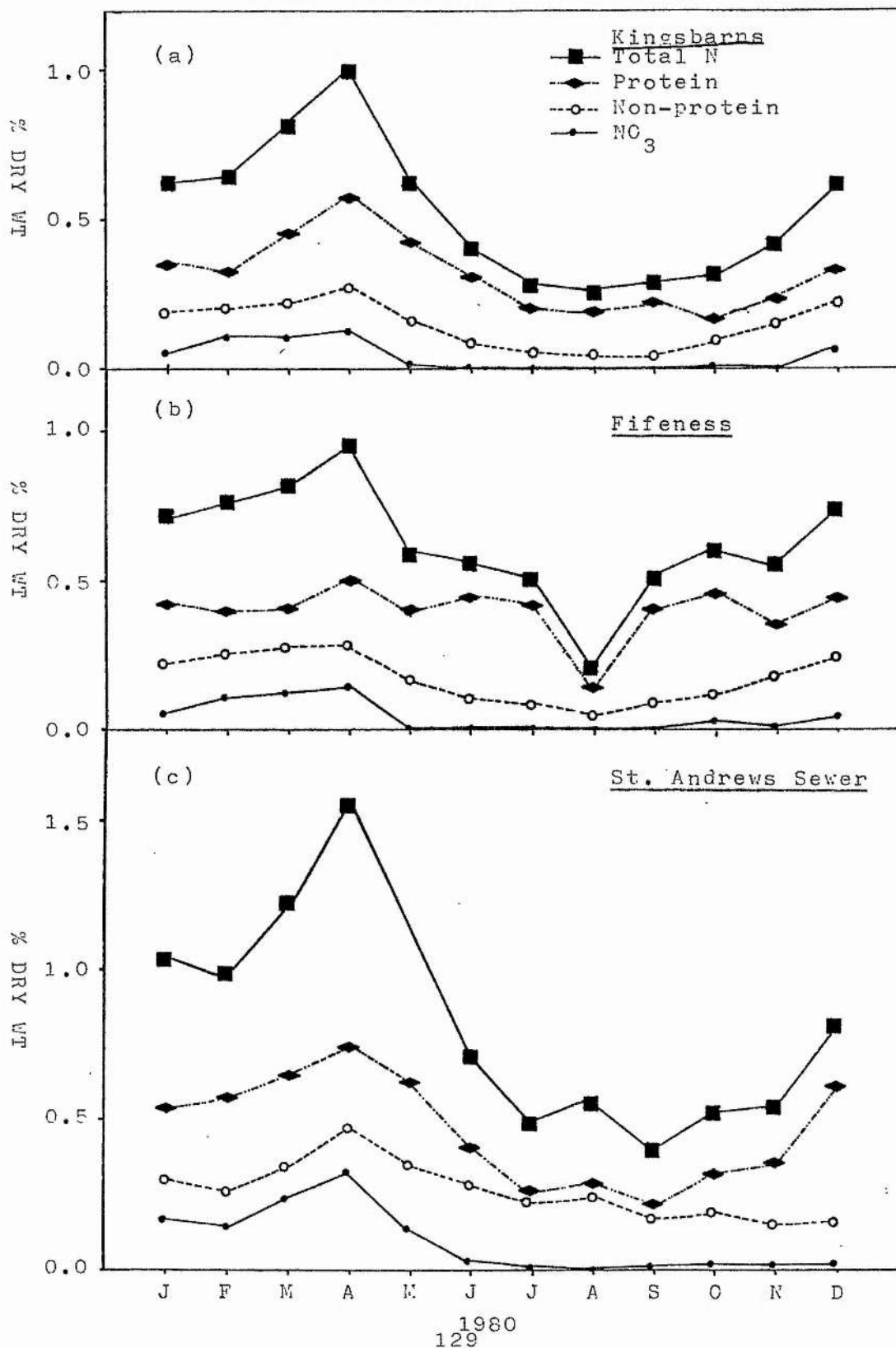


Table 4 iv b. Internal N content (% dry wt) of mature frond tissue of L. digitata from the 4 sampling sites.

1980	Site	Total N	Protein	Non-Protein	Inorganic-N
Jan	KB	0.54	-	-	-
	FN	0.61	-	-	-
	St. A	1.49	0.99	0.44	0.06
	SEW	1.75	-	-	-
Apr	KB	0.90	-	-	-
	FN	0.93	-	-	-
	St. A	3.06	1.56	1.29	0.21
	SEW	3.38	-	-	-
July	KB	0.89	0.82	0.07	0.00
	FN	1.01	0.95	0.06	0.00
	St. A	1.72	1.33	0.33	0.01
	SEW	1.31	1.03	0.28	0.01
Sept	KB	0.73	0.65	0.08	0.00
	FN	0.56	0.49	0.07	0.00
	St. A	0.66	0.44	0.22	0.00
	SEW	0.54	0.37	0.17	0.00
Oct	KB	0.52	0.37	0.15	0.00
	FN	0.59	0.47	0.12	0.00
	St. A	0.79	0.58	0.21	0.01
	SEW	0.x5	0.51	0.13	0.00

than at Kingsbarns and Fifeness. In the meristem this distinction is maintained throughout the year (Fig. 4 xi(a-c)) whereas, in the mature tissue (Table 4 iv b) by late summer all 4 sites have a similar internal N content.

By comparing the ratios of the total N content (at the April maximum) to the external N concentration of the seawater at the 4 sites, it is possible to establish whether the differences in tissue N content at the sites merely reflects differences in seawater nitrogen concentrations. The mean winter seawater N concentration ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4$) Total N and the ratio of $\frac{\text{Total N}}{\text{External N Conc.}}$ for the 2 species in the meristem and mature frond tissue is shown in Table 4 v. If the tissue N content is directly proportional to the seawater N concentration then the values obtained at the different sites will be similar.

Table 4 v. Total internal N/seawater N concentration at the 4 sampling sites for L. saccharina and L. digitata (meristem and mature frond tissue).

Site	Ext* N	<u>L. saccharina</u>		<u>L. digitata</u>	
		Meristem	Mature	Meristem	Mature
KB	14.32	96.81	72.22	69.57	63.07
FN	15.39	90.79	76.55	61.41	63.75
St. A	17.94	76.78	187.89	70.23	170.39
SEW	24.17	70.14	145.12	64.18	139.86

* External N concentration $\mu\text{g-at N.l}^{-1}$

KB=Kingsbarns

FN=Fifeness

St. A=St. Andrews

SEW=Sewer

Values for the 4 sites are similar in the meristem of L. digitata. However, in L. saccharina meristem, Kingsbarns and Fifeness have similar values as do Sewer and St. Andrews, but the former 2 sites had a relatively higher N content than would be expected simply from the external concentration. The higher degree of exposure and therefore, greater water turbulence at Fifeness may suggest increased availability of nutrients and hence, increased uptake and a higher internal N content, but Kingsbarns and St. Andrews are subjected to a similar degree of exposure and this does not provide an adequate explanation.

In the mature tissue of both L. saccharina and L. digitata, St. Andrews and Sewer have a proportionally higher N content than is predicted simply from the supply concentration. This difference may indicate the non-linear uptake of N with concentration or that at high N concentrations uptake shifts to a second (upper phase) and accumulation then proceeds at a much faster rate (see later, 4 iv).

c Differences in Seasonal Variation between mature and meristematic tissue

L. saccharina. Total N is maximal in April in both the meristem and the mature tissue at St. Andrews (Figs 4 vi-vii) but the N content of the meristem drops much more rapidly than in the mature tissue after the April peak. In the meristem, protein and non-protein N occur at approximately similar levels throughout the year, in contrast to the

mature tissue where the protein content is always considerably higher than non-protein N. Inorganic-N shows a similar seasonal variation in both frond areas but in the meristem nitrate comprises a much greater proportion of the total N during January-April than in mature tissue.

L. digitata. The seasonal variation of internal N is similar in both frond areas (Figs 4 viii+ix) but like L. saccharina the N content of the meristem is only about $\frac{1}{2}$ that of the mature tissue. There is also more non-protein relative to protein in the mature than meristematic tissue.

The mature tissue, unlike the meristem, is no longer undergoing cell division and cell enlargement, utilisation of N is therefore, probably minimal and N then accumulates to a greater extent than in the meristem where continuous utilisation for growth and dilution by N-free tissue would lower the relative N content of this frond area.

d Variation between species

The seasonal variation of total N and the relative N content at St. Andrews is similar for L. saccharina and L. digitata. In April the total N content of the mature tissue is 3.06% dry wt in L. digitata and 3.37% dry wt in L. saccharina, and in the meristem, 1.26% and 1.22% respectively. Protein and non-protein N content is similar in the mature tissue but L. digitata has a lower inorganic-N content than L. saccharina. In the meristematic tissue, inorganic-N is proportionally less important in L. digitata than L. saccharina from January-April, with protein comprising

a much greater proportion of the total-N during this period.

e Relative Proportions of Protein, Non-protein and Inorganic-N

In the mature tissue of both species, protein and non-protein occur in similar proportions relative to inorganic-N (Table 4 vi).

Table 4 vi. The relative proportions of the 3 nitrogen fractions in the mature (Mat) and the meristematic (Mer) frond tissue of L. digitata and L. saccharina in April at St. Andrews.

Protein : Non-Protein : Inorganic						
<u>L. saccharina</u>	Mer	0.4	:	0.5	:	1.0
	Mat	4.6	:	3.2	:	1.0
<u>L. digitata</u>	Mer	3.6	:	1.6	:	1.0
	Mat	6.1	:	7.4	:	1.0

The relative proportions of the 3 N forms in the meristem are very different between species with protein comprising the least and inorganic-N the greatest proportion in L. saccharina but the reverse is true of L. digitata. However, since relative proportions vary with season few conclusions can be made from this table. Perhaps a better measure of relative proportions is obtained by calculating the ratio of $\frac{\text{Protein}}{\text{Non-Protein} + \text{Inorganic-N}}$ as a measure of consistency between protein and the N forms which may be

Fig. 4 xiii.

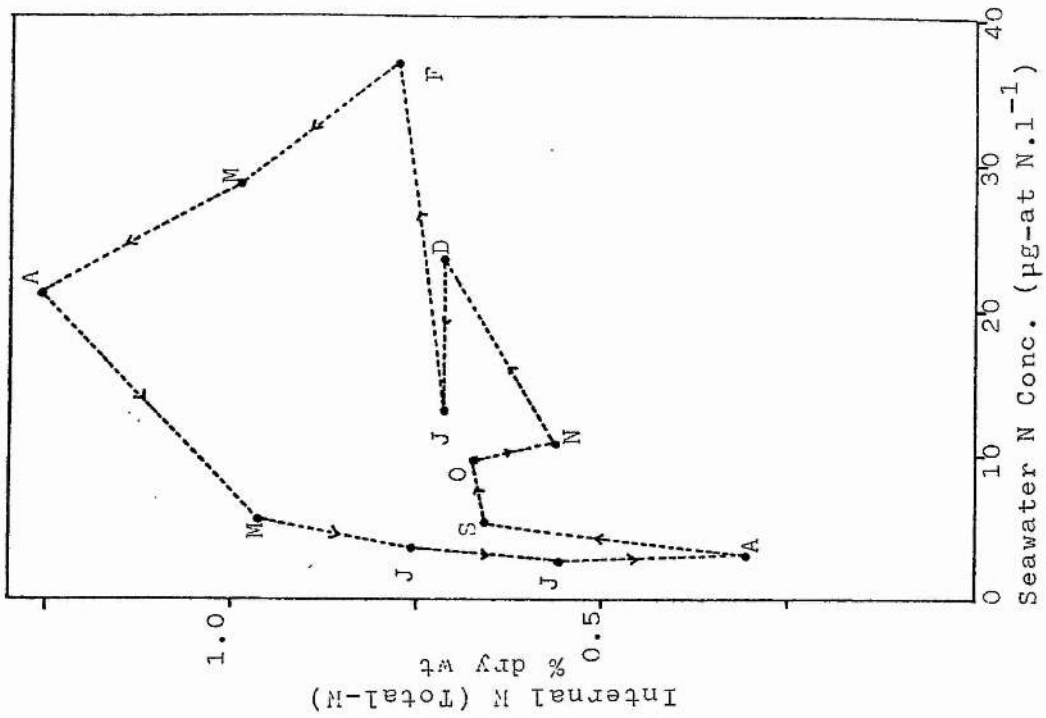
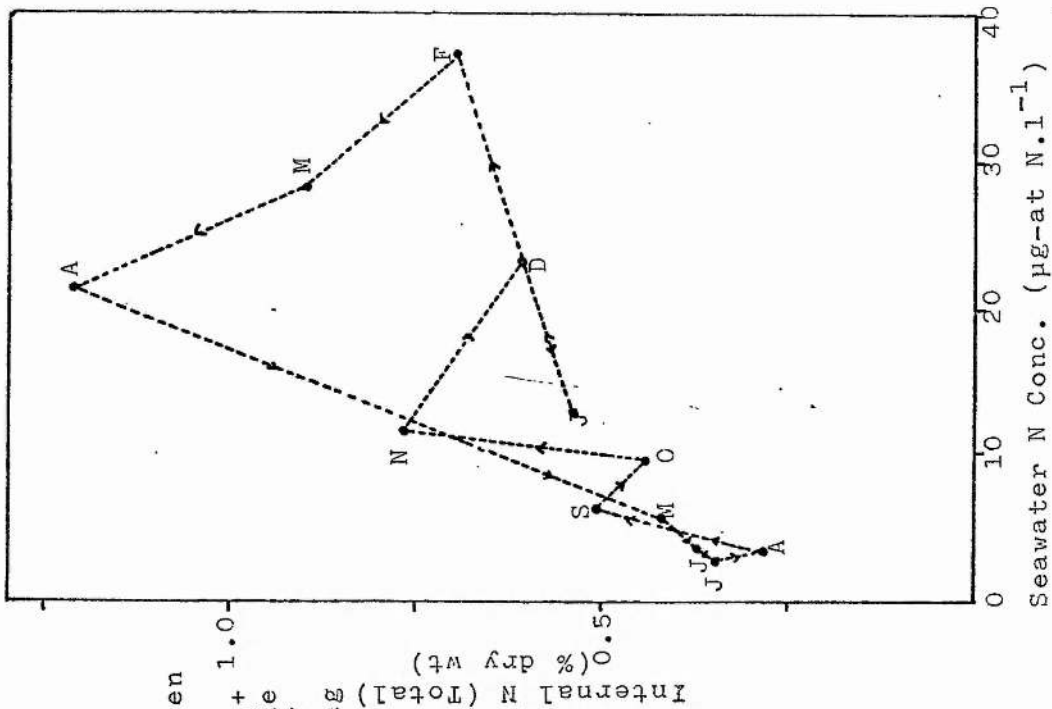


Fig. 4 xii.



Relationship between seawater nitrogen concentration ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4$) and tissue total-N content of the meristem during 1980 in

L. saccharina

(Fig. 4 xii)

and

L. digitata

(Fig. 4 xiii).

synthesised into protein in the frond. Such a calculation shows that in the meristem and mature frond tissue of L. digitata and in the meristem of L. saccharina, the ratio increases gradually during the year. Protein, therefore, increases relative to the other 2 fractions, possibly increasing as a result of utilisation for protein synthesis of the non-protein and inorganic-N fractions. In the mature tissue of L. saccharina, however, (with the exception of high values for June and August) the ratio is relatively constant at 1.5; the protein content is therefore, maintained as a consistent proportion of the total N of the frond.

iii CORRELATIONS BETWEEN SEAWATER N, INTERNAL N AND GROWTH

a The effect of exogenous N on internal N

In the meristem of L. saccharina (Fig. 4 xii) seawater N ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4$) and internal N (Total N) shows an overall positive linear correlation from December-February and April-November. This latter correlation is highly significant ($P < 0.001$). In L. digitata (Fig. 4 xiii) meristem a similar positive correlation exists for most of the year (May-August and September-February). Although a significant linear correlation does not necessarily indicate a direct causal relationship, the internal N content appears to follow changes in seawater N concentration during most of the year. However, as discussed previously (4 ii b), the tissue N content is not simply determined by the external

Figs 4 xiv and xv. Relationship between linear growth rate of the frond and seawater nitrogen concentration ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4$) of L. digitata (Fig 4 xiv) and L. saccharina (Fig 4 xv) at St. Andrews during 1980.

Fig 4 xiv.

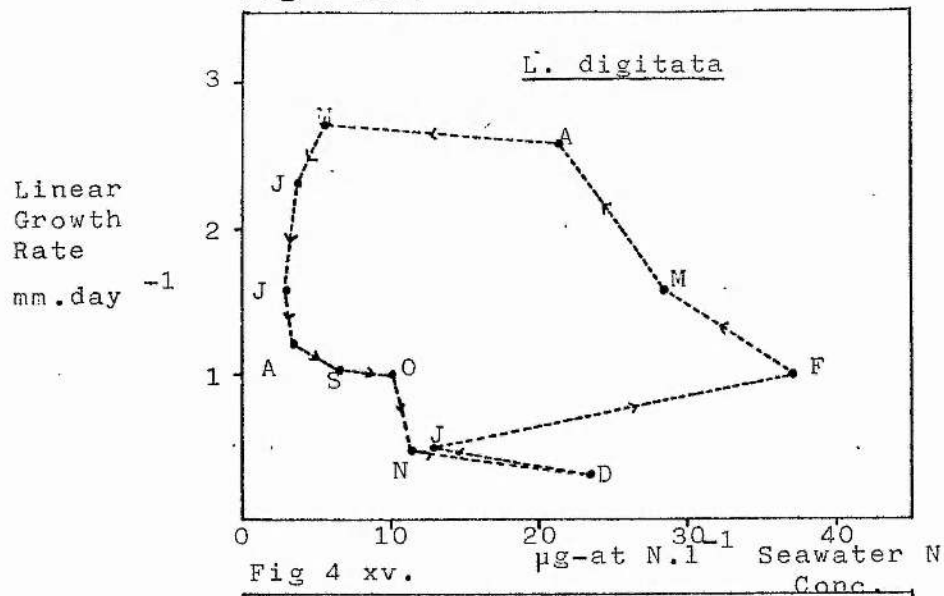


Fig 4 xv.

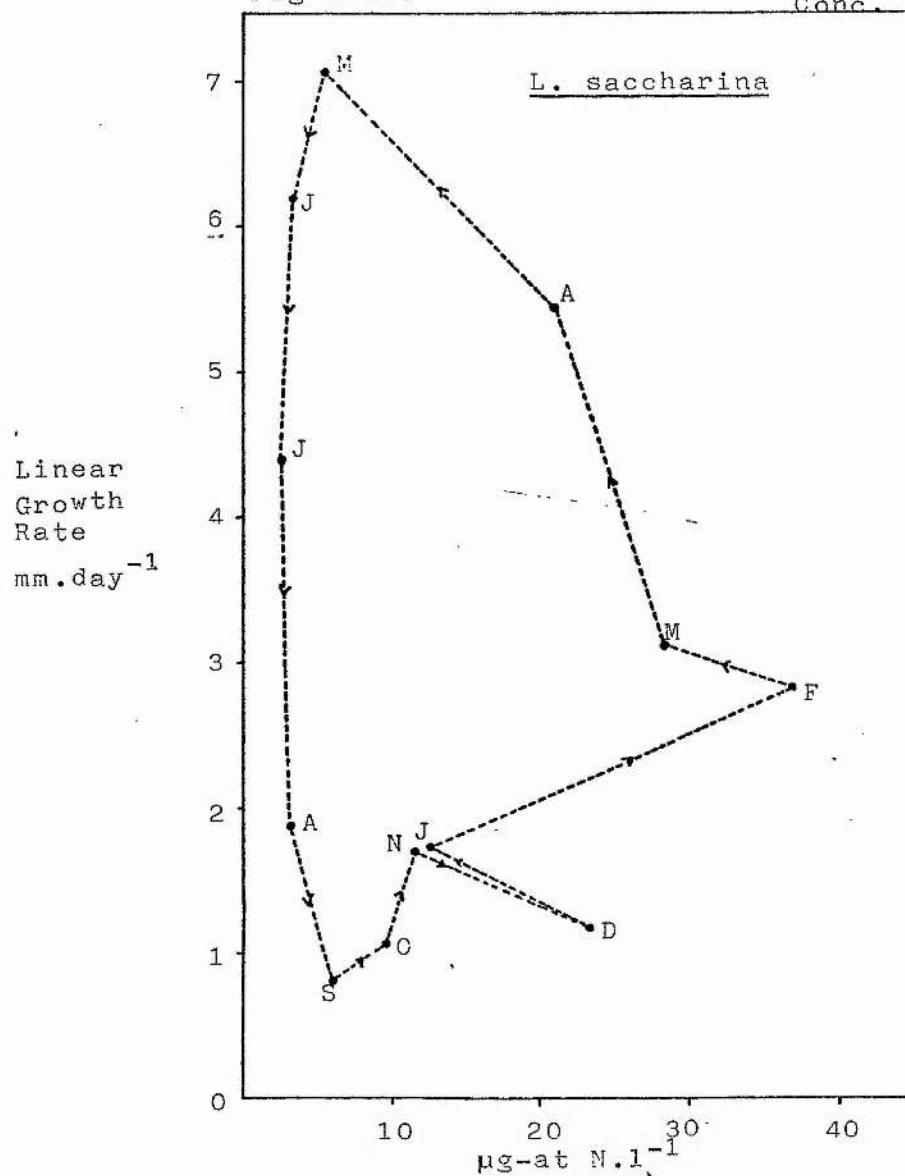


Fig 4 xvi. *L. saccharina*

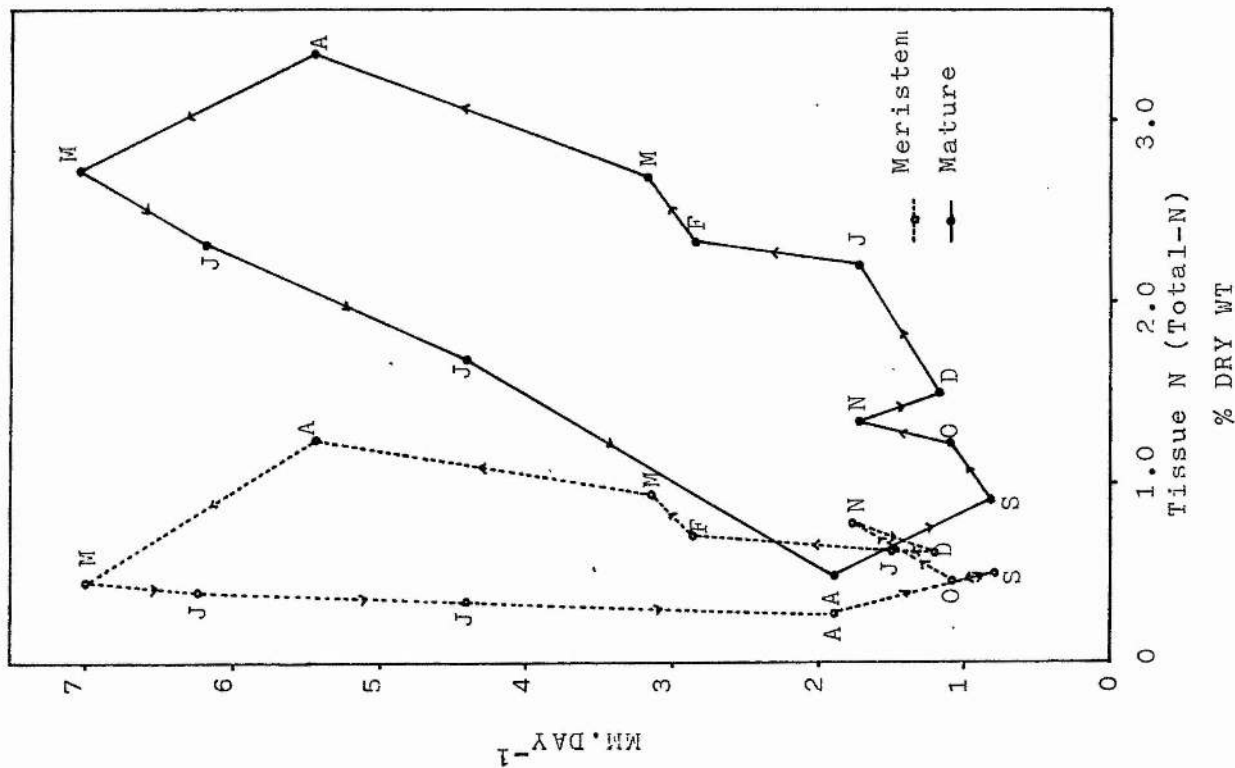
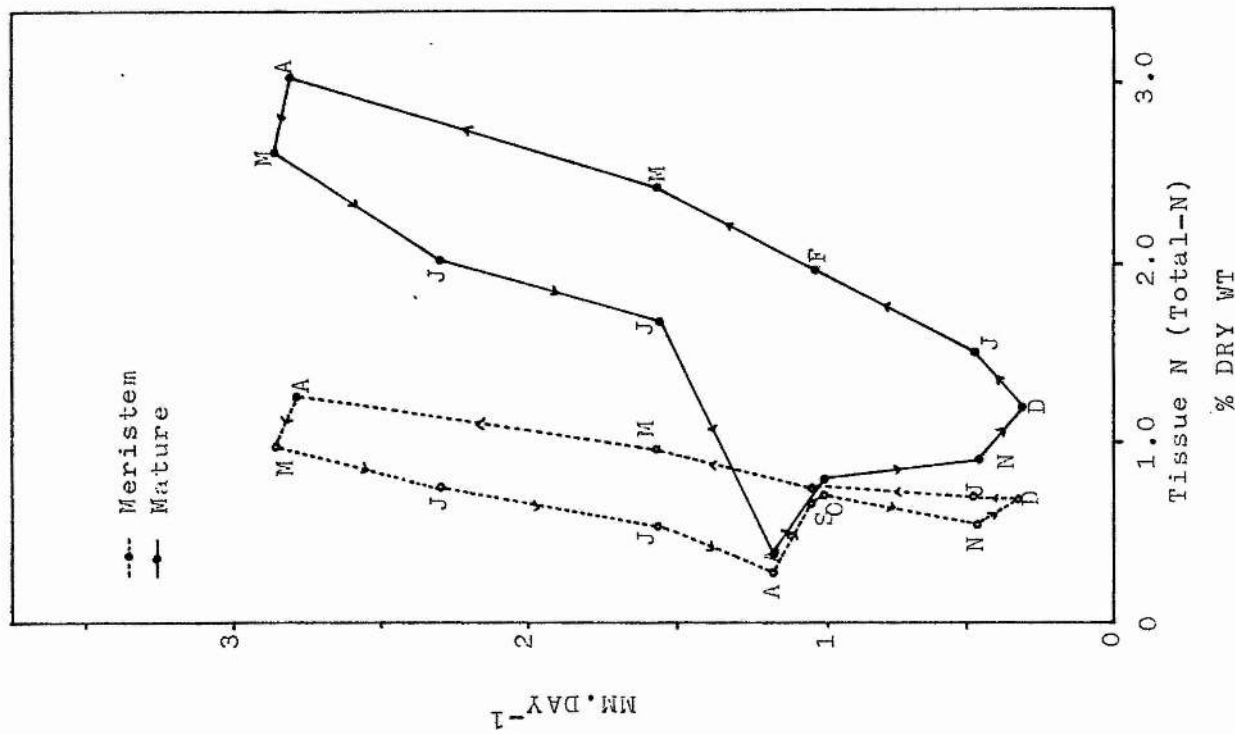


Fig 4 xvii. *L. digitata*



N concentration and other factors *es.* exposure and water turbulence are probably important.

b Exogenous N and Growth

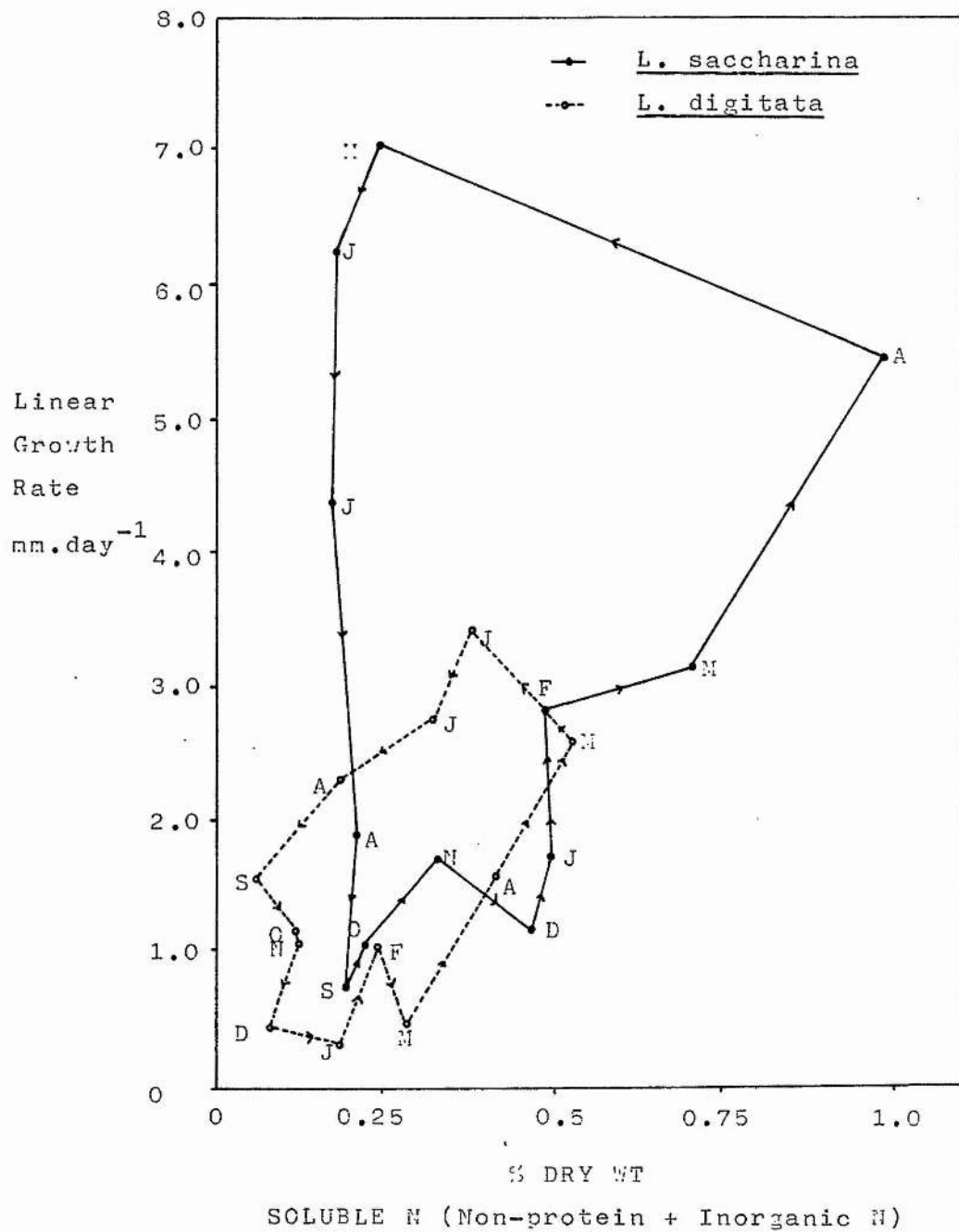
Growth rates of L. saccharina at St. Andrews (Fig. 4 xv) shows an overall positive correlation from May-August and from September-February. During February-May these factors are inversely related ($P < 0.10$). L. digitata also shows a negative correlation from February-May (Fig. 4 xiv) but from August-January the relationship is also a negative one ($P < 0.10$). Exogenous N and growth are only positively correlated during May-August when growth rate declines as seawater N declines.

The apparent anomaly of maximum growth (May) at a time of minimum seawater N suggests that there is probably a time lag between uptake of N from the seawater and utilisation for growth by the alga. The correlation of growth rate and internal N would then give a better indication of the relationship of nitrogen and growth.

c Internal N and Growth

In L. saccharina (Fig. 4 xvi) growth rate and internal N show a positive linear relationship from December-April ($P < 0.02$) and May-August ($P < 0.01$) in the mature tissue. The relationship is similar in L. digitata (Fig. 4 xvii) with significant positive linear correlations occurring during September-April ($P < 0.02$) and May-August ($P < 0.05$) in the meristem and December-April ($P < 0.01$) and May-November ($P < 0.05$) in the mature tissue. Growth then tends

Fig 4 xviii. Relationship between soluble-N content of the meristem and frond growth rate of *L. saccharina* and *L. digitata* at St. Andrews during 1980.



to follow changes in internal N content throughout most of the year, increasing as internal N increases and slowing as internal N decreases.

It is probably better to consider growth rate in relation to the readily accessible and utilisable tissue N (low molecular weight organics (non-protein) and inorganic-N). The same positive relationship is exhibited in the meristem of both L. saccharina and L. digitata (Fig. 4 xviii), with linear relationships in L. saccharina from September-April and May-October and in L. digitata from January-May and June-December.

At Sewer and St. Andrews the ratio of the mean nitrate concentration of the seawater during the winter months is $\frac{20.22}{15.87} = 1.27$.

In L. digitata, the ratio of internal N (total N) at the 2 sites is $\frac{1.12}{0.96} = 1.17$.

The 2 values are very similar as is expected, since total N content of the frond is positively correlated with the seawater N concentration.

The ratio of growth rates (Maximum growth rates in May) of L. digitata at the 2 sites is $\frac{6.308}{2.741} = 2.301$.

If the growth rate was simply controlled by the level of nitrate in the seawater and hence the N content of the frond this value would be similar to the ratio of seawater nitrate concentration and the ratio of internal N content at the 2 sites. Perhaps this discrepancy can be explained in terms of the ability of Laminaria to take up and utilise

all 3 nitrogen sources (nitrate, nitrite and ammonium) simultaneously (see later, 4 iv f). However, the discrepancy is still observed when the ratio in growth rates (2.30) is considered with the ratio of the mean concentration of N ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4$) in the seawater at the 2 sites $\frac{24.17}{17.94} = 1.35$. Hence, growth rate is not directly determined by total available external soluble N concentration since a small rise in external N concentration (ie. 24.17 $\mu\text{g-at N.l}^{-1}$ at the Sewer site compared to 17.94 $\mu\text{g-at N.l}^{-1}$ at St. Andrews) results in a greatly increased growth rate. Such a discrepancy also exists with L. saccharina between Sewer and St. Andrews, where growth rates are in the ratio 1.81, the ratio of external nitrate is 1.27 and the ratio of internal (total) N is 1.39 and the total external N ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4$) is 1.35.

Therefore, Laminaria growth rates are not simply determined by the seawater and tissue N content but, in addition, some other factors eg. turbulence, phosphate concentration and the period of emersion may all be important and are discussed in more detail later.

iv UPTAKE OF NITROGEN

The following section is concerned with uptake of nitrogen which is measured as loss of nitrogen from the medium (and in some cases also increase in nitrogen by the tissue). In order to draw any conclusions from the data collected it must be ensured that the nitrogen lost from the medium is actually taken up by Laminaria and not

by epiphytes on the algal surface. Adequate cleaning of the algal surface is, therefore essential. In most previous Laminaria studies, the frond surface is cleaned by wiping (e.g. Kain, 1963) but no attempt appears to have been made to ensure that this method is adequate.

a Cleaning of Laminaria for Growth and Uptake Experiments

Electron-micrographs were taken of the surface of the frond, stipe and holdfast of L. saccharina and L. digitata from plant material which had:

- i not been cleaned or 'untreated'
- ii Cleaned by placing under 10 l of running filtered seawater
- iii Cleaned by wiping with muslin

The surface of the untreated tissue was seen to be densely covered in bacteria, relatively uniform in shape and size and presumably adhering to the mucilage on the tissue surface (Figs 4 xix a+b). The epiphytes consisted almost entirely of these bacteria although the occasional pennate diatom was observed. The bacteria covered the whole sporophyte (frond, stipe and holdfast) of both species.

Cleaning in running filtered seawater was ineffective and there was no significant difference between the untreated and tissue treated in this way. This was probably because the running water did not remove the surface mucilage and hence the bacteria adhering to it.

However, wiping with muslin (Figs 4 xx a+b) was very

Fig 4 xix a + b.

Electron Micrographs of the 'untreated' frond surface of L. saccharina showing the covering of bacteria adhering to the mucilage.

Fig 4 xix a. Magnification X2,000

Fig 4 xix b. Magnification X5,000

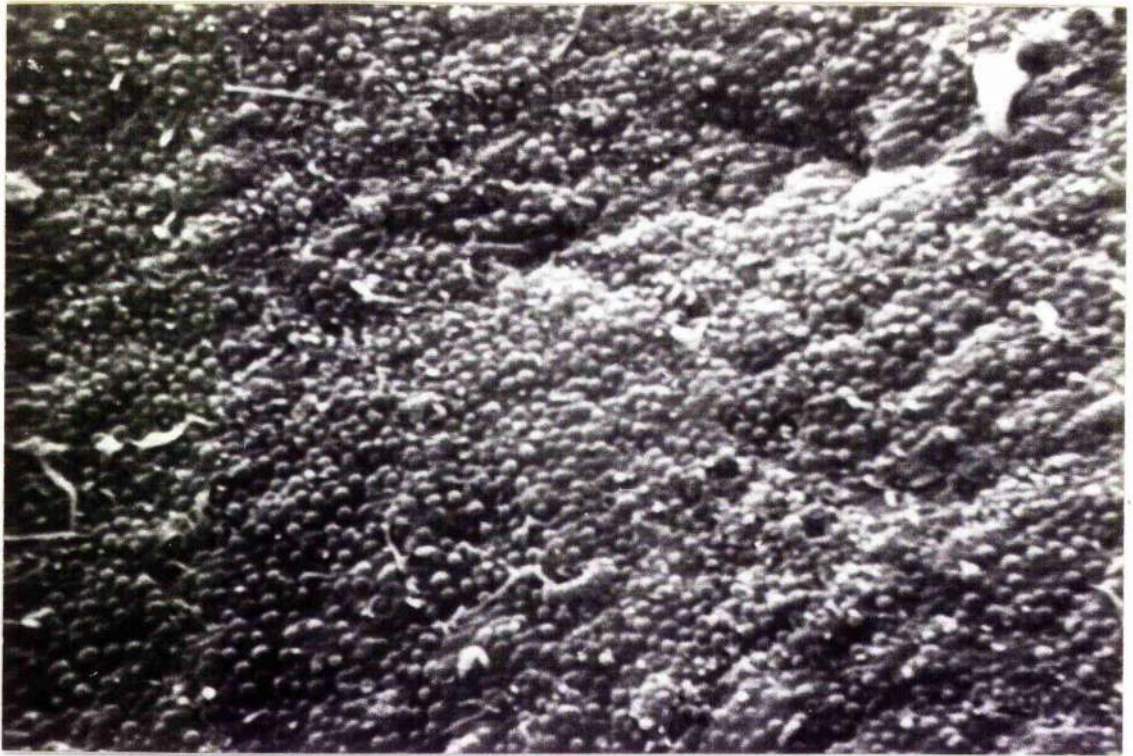


Fig 4 xix a.

10 μ

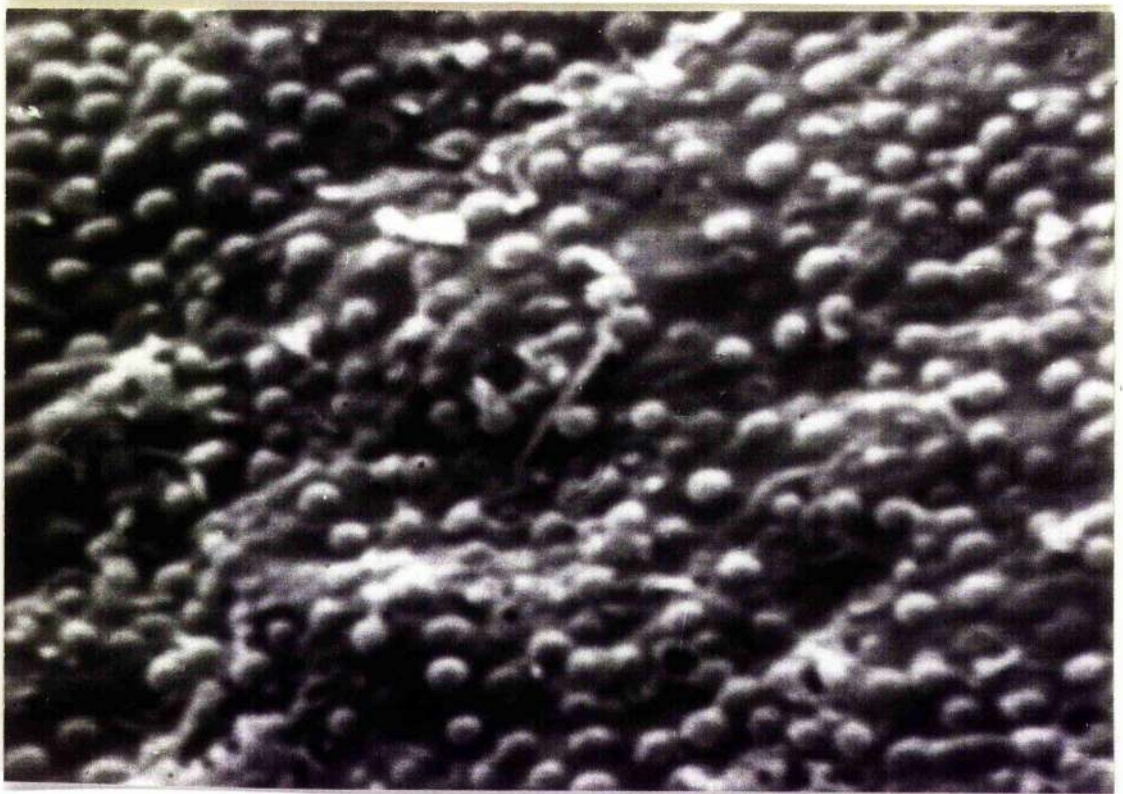


Fig 4 xix b.

4 μ

Fig 4 xx a + b.

Electron Micrographs of the frond surface of L. saccharina after cleaning by wiping with muslin to remove the adherent bacteria.

Fig 4 xx a. Magnification X2,000

Fig 4 xx b. Magnification X2,000

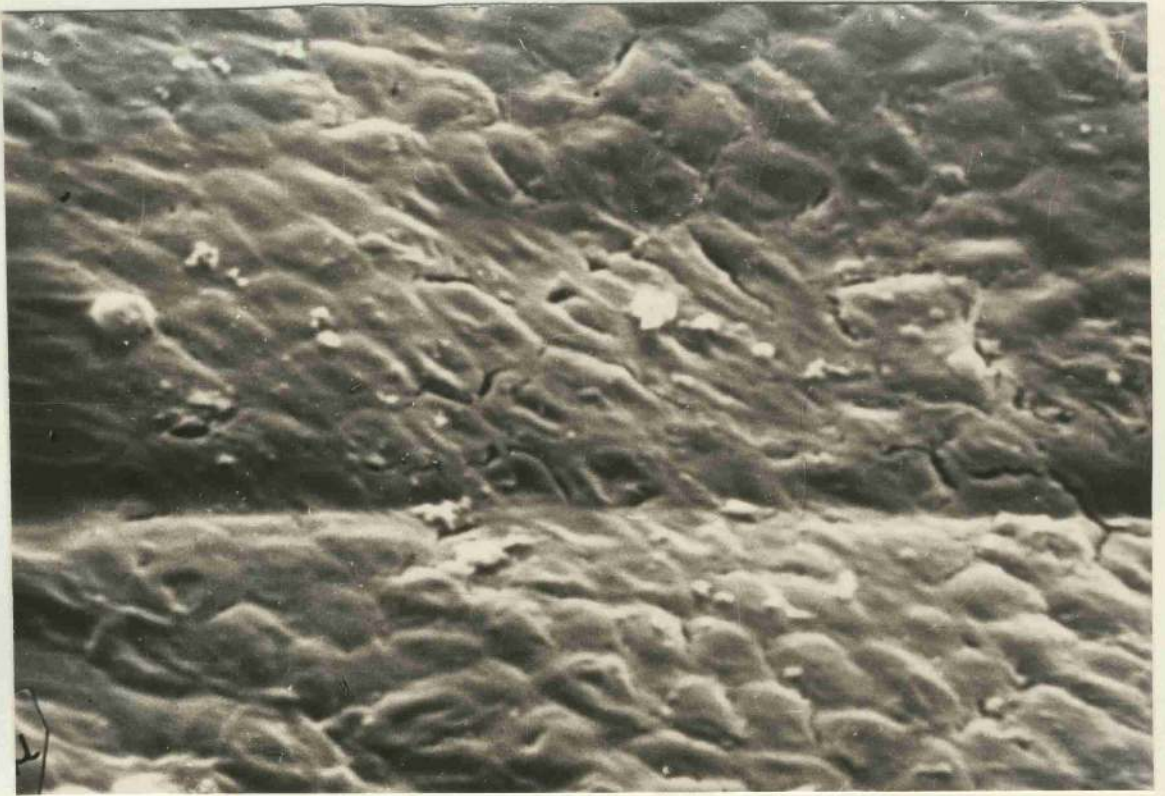


Fig 4 xx a.

10 μ



Fig 4 xx b.

10 μ

The effect of concentration on uptake of nitrate by discs of L. digitata over 5 hours in the light.

Fig 4 xxi a.

Uptake of nitrate by discs of L. digitata over 5 hours at 10 °C in saturating white light with high phosphate concentration (3.0 $\mu\text{g-at P.l}^{-1}$). Mean rate of uptake \pm SE with 3 replicates/treatment.

Fig 4 xxi b.

Lineweaver-Burke transformation of uptake of nitrate by discs of L. digitata (see legend Fig 4 xxi a) between 0 - 31.6 $\mu\text{g-at N.l}^{-1}$ external concentration.

Fig 4 xxi a. (Legend on facing page)

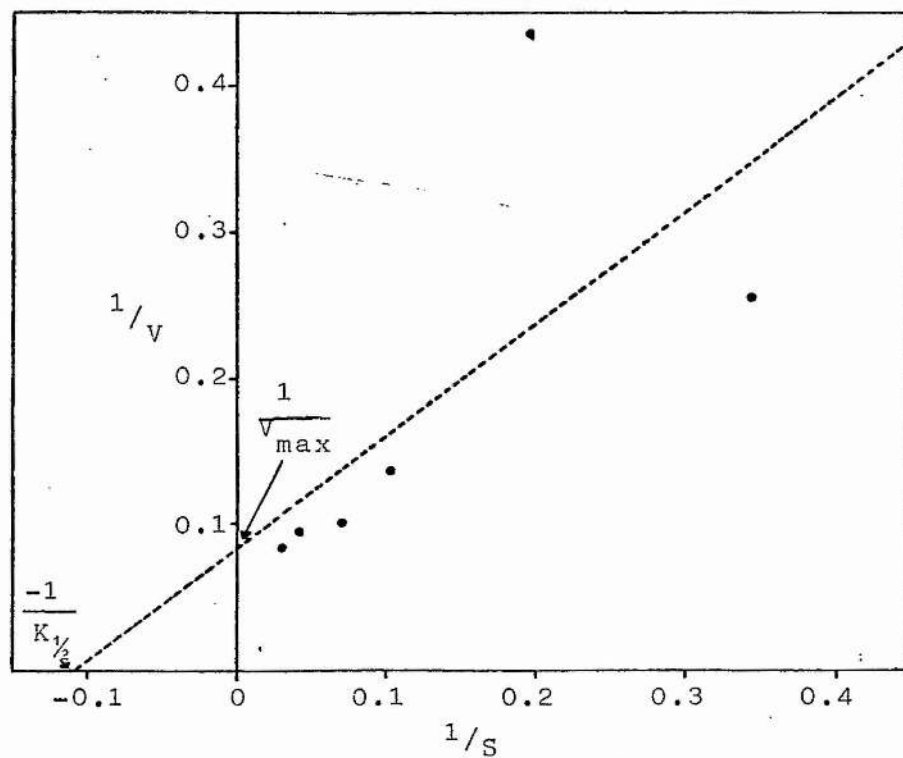
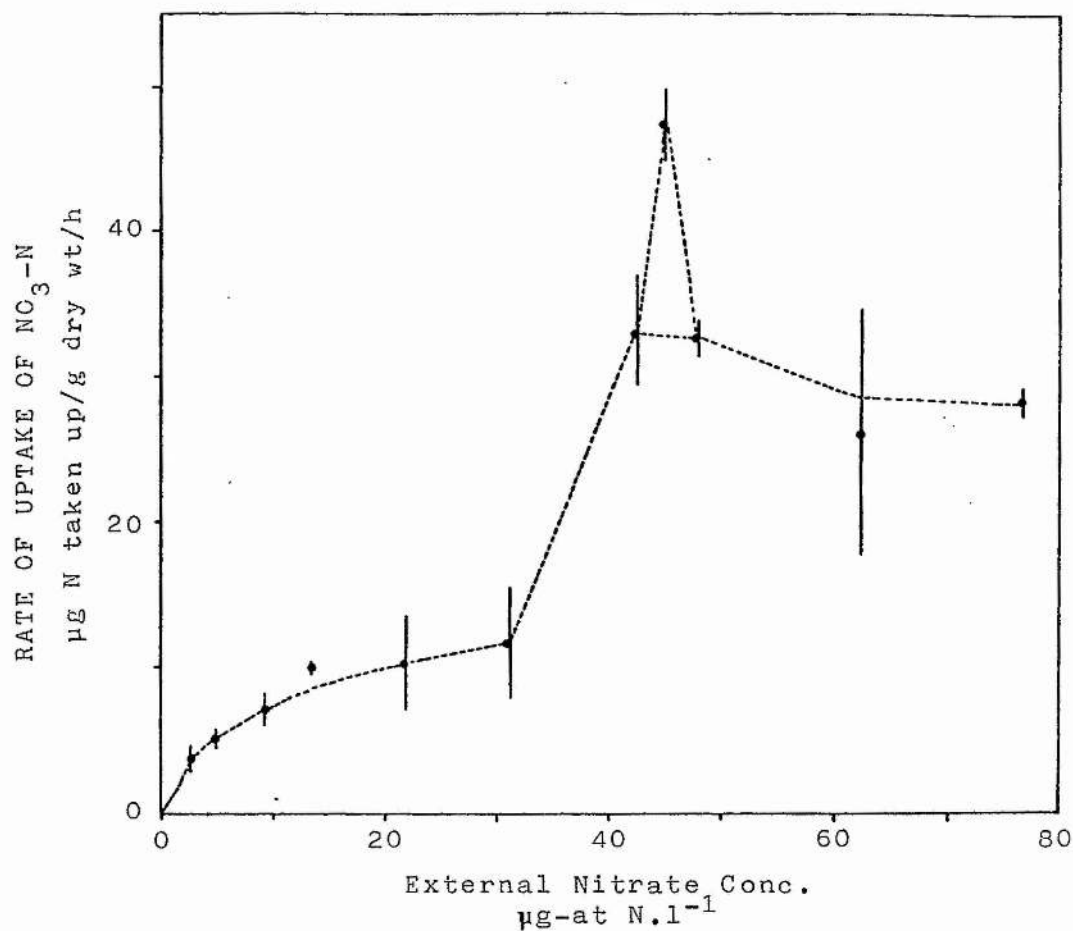


Fig 4 xxi b. (Legend on facing page)

effective and virtually all the epiphytes were removed, from the frond, stipe and holdfast surface of both L. digitata and L. saccharina.

Therefore, cleaning the algal surface by wiping with muslin does effectively remove the epiphytes and accordingly this method was used to clean the Laminaria for all uptake and growth experiments undertaken in this study. During growth experiments the tissue, either discs of frond tissue or whole plants were cleaned every 2 days (whilst being measured) to prevent those epiphytes remaining from building up to any great extent and therefore, reducing the availability of nutrients for Laminaria growth.

b The effect of concentration on uptake of nitrate by discs of L. digitata in the light

Uptake of nitrate by discs of L. digitata over 5 hours at 10 °C in saturating white light with high phosphate concentration (3.0 $\mu\text{g-at P.l}^{-1}$) (Fig. xxi a).

Uptake shows an Epstein 2 phase plot; uptake follows saturation kinetics from 0 to about 32 $\mu\text{g-at N.l}^{-1}$ (first phase) then increases steeply to a relatively stable rate of 32 $\mu\text{g N.g dry wt}^{-1}.\text{h}^{-1}$ at a substrate concentration of 42 $\mu\text{g-at N.l}^{-1}$. Using a Lineweaver Burke Plot of $1/V$ vs $1/S$ where V = uptake velocity and S = substrate concentration estimates for the kinetic parameters K_m and V_{max} were obtained (Fig. 4 xxi b). During the first phase uptake is saturated between 22-32 $\mu\text{g-at N.l}^{-1}$;

$$\begin{aligned} V_{\text{max}} &= 12.05 \mu\text{g N.g dry wt}^{-1}.\text{h}^{-1} \\ K_m &= 9.26 \mu\text{g-at N.l}^{-1} \end{aligned}$$

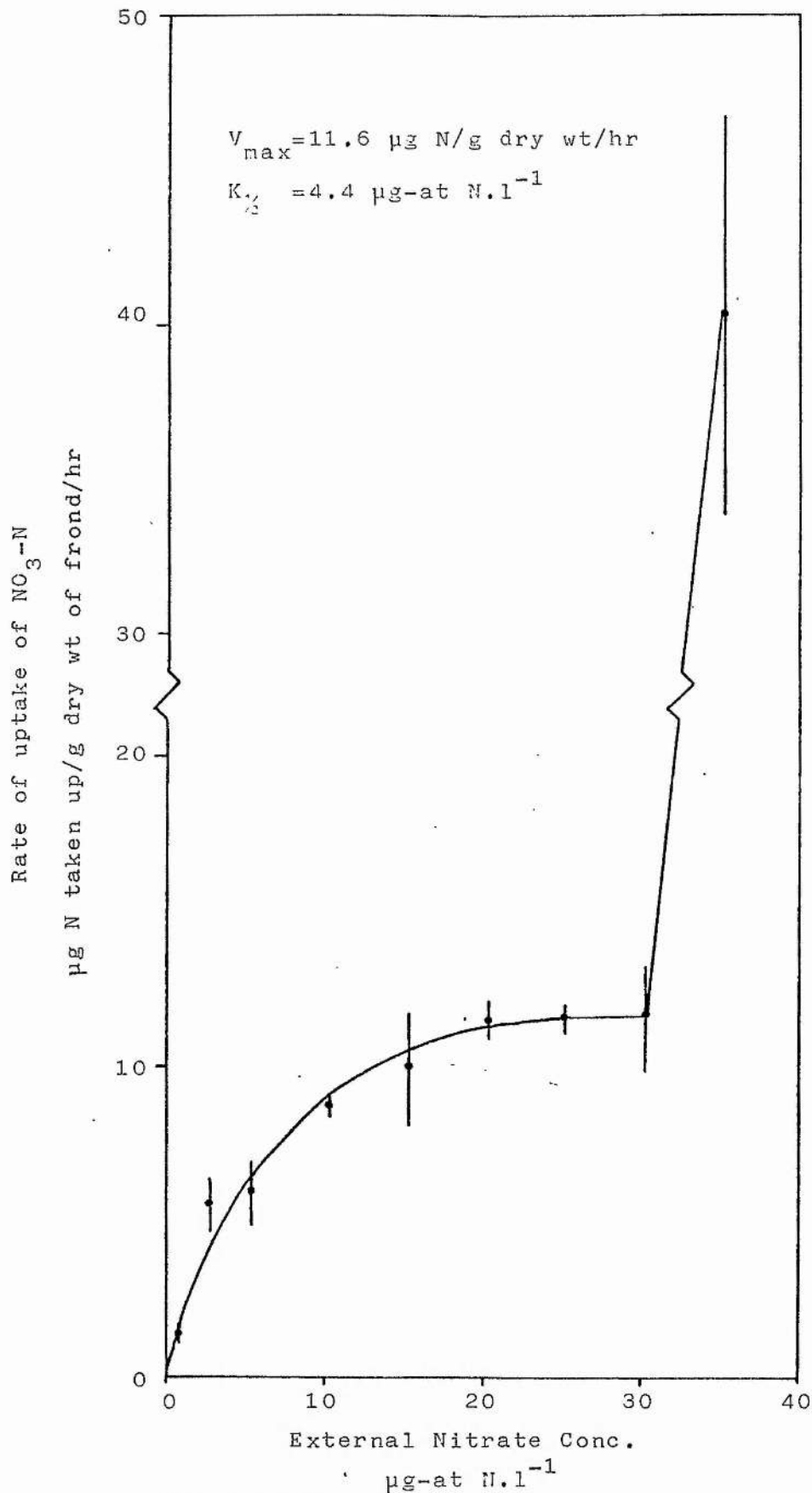


Fig 4 xxii.

Uptake of nitrate by whole plants of *L. saccharina* over 3 hrs at 10°C in saturating white light with high phosphate ($3.0 \mu\text{g-at P.l}^{-1}$). Mean rate \pm SE with 3 replicates/treatment. (Rate of uptake measured as $\mu\text{g N taken up/g dry wt of frond/hr}$.)

The value at $45.33 \mu\text{g-at N.l}^{-1}$ of $47.55 \pm 2.45 \mu\text{g N.g dry wt}^{-1}.\text{h}^{-1}$ is very high and may result from a technical error. It is therefore, ignored.

c The effect of concentration on the uptake of nitrate by whole plants of *L. saccharina* in the light

Uptake of nitrate over 3 hours by whole plants of *L. saccharina* at 10°C in saturating white light with high phosphate ($3.0 \mu\text{g-at P.l}^{-1}$) (Fig. 4 xxii).

Uptake follows a similar pattern to that shown by discs of *L. digitata*. Uptake shows an Epstein 2 phase plot; during the first phase uptake follows saturation kinetics up to a substrate concentration of $25 \mu\text{-at N.l}^{-1}$; the rate of uptake then increases rapidly into the 2nd phase after $30 \mu\text{-at N.l}^{-1}$.

During the first phase,

$$V_{\text{max}} = 11.6 \mu\text{g N.g dry wt of frond}^{-1}.\text{h}^{-1}$$

$$K_{1/2} = 4.4 \mu\text{g-at N.l}^{-1}$$

d Time Course Uptake of nitrate by discs of *L. digitata* over 24 hours in the light

Fig. 4xxiii shows there is an initial rapid loss of nitrate from the medium during the first 3 hours but after this uptake proceeds at a steady rate ($6.42 \mu\text{g N lost.g dry wt}^{-1}.\text{h}^{-1}$). The mean rate of uptake over 24 hours is $12.95 \pm 0.79 \mu\text{g N.g dry wt}^{-1}.\text{h}^{-1}$ and $266.6 \mu\text{g N}$ were lost from the medium during the course of the experiment.

The nitrate (inorganic-N) content of the tissue (Fig. xxiii) increases slightly over the first 15 hours but increases

Time Course Uptake of Nitrate by discs of L. digitata over 24 hours.

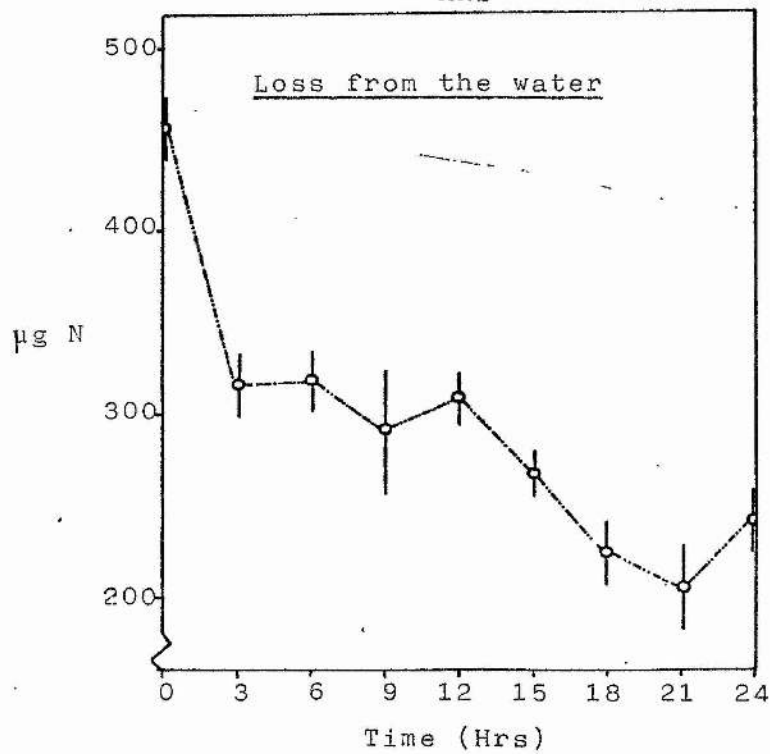
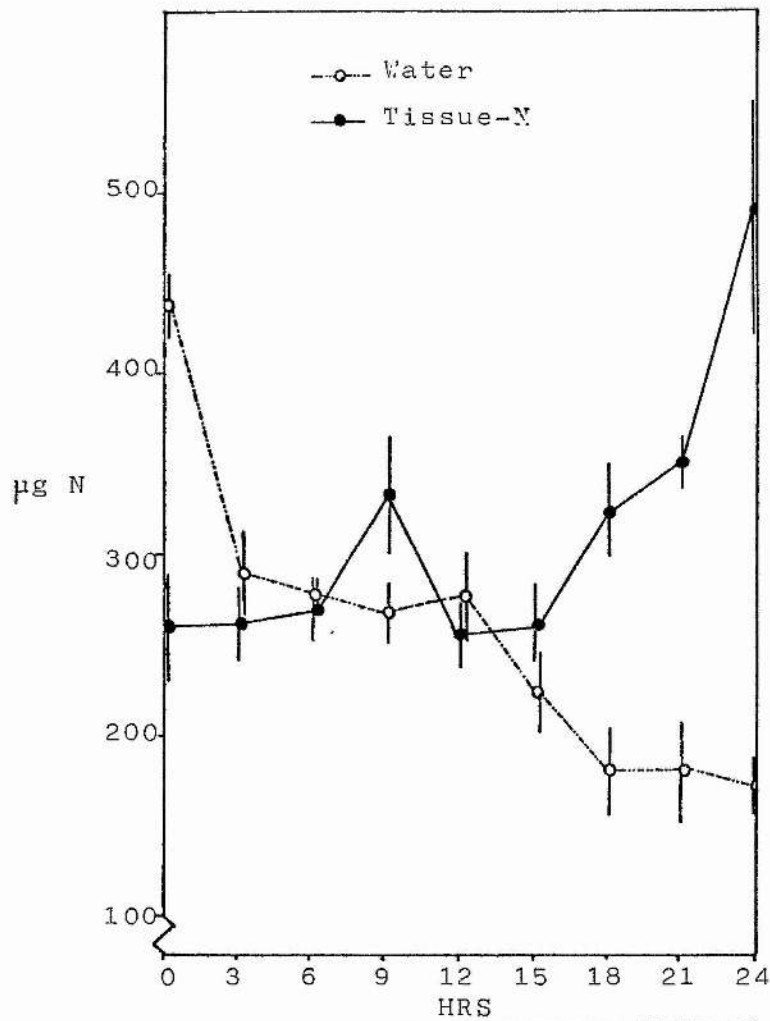
Fig 4 xxiii.

Time course uptake of nitrate by discs of L. digitata over 24 hours at 10 °C in saturating white light with high phosphate concentration (3.0 $\mu\text{g-at P.l}^{-1}$). Initial nitrate concentration of the medium was 31.5 $\mu\text{g-at N.l}^{-1}$. Graph shows loss of N from the medium and gain of N (inorganic-N) by the tissue.

Fig 4 xxiv.

Time course uptake of nitrate by discs of L. digitata in the dark. See legend of Fig 4 xxiii for details.

Fig 4 xxiii. (See facing page for legend).

Fig 4 xxiv.
(See facing page for legend)

rapidly after this. The total increase in tissue nitrate content is $260.3 \pm 1.3 \mu\text{g N}$ and the mean rate of increase over 24 hours was $12.64 \pm 1.49 \mu\text{g N.g dry wt}^{-1}.\text{h}^{-1}$.

The uptake rates and the amount of N lost from the water and extractable from the tissue are not significantly different, suggesting that most of the nitrate taken up by the tissue (which was not N-limited prior to the experiment) is retained as inorganic nitrate and is not reduced further over this short-term experiment. There is no obvious explanation as to why the initial rapid loss from the water (0-3 hours) is not detected as a significant increase in tissue N during the same period. It is possible that nitrate is absorbed initially very rapidly onto the mucilage film over the disc surface, and that the mucilage is lost with its adherent N during extraction of N and hence it is not detected in this first reading.

e Time Course Uptake of Nitrate by discs of
L. digitata over 24 hours in the dark

The pattern of loss from the water in the dark is very similar to that in the light (Fig. 4 xxiv) with an initial rapid loss from the medium (0-3 hours) followed by a relatively constant loss from 3-24 hours. The mean rate of loss from the medium over 24 hours was $10.45 \pm 0.84 \mu\text{g N.g dry wt}^{-1}.\text{h}^{-1}$. The rate of uptake was, therefore, significantly reduced in the dark ($P < 0.10$) to 30.7% of the uptake rate in the light.

Since little of the nitrate taken up in the light is

Table 4 vii. Simultaneous Uptake of nitrate, nitrite and ammonium by discs of *L. digitata* supplied at concentrations of 7.5, 1.5 and 3.0 μM -at N.l^{-1} respectively. Uptake ($\mu\text{g N.g dry wt}^{-1}.\text{h}^{-1}$) was measured over a period of 5 hours. (Mean \pm SD with 3 replicates/treatment).

Treatment	Rate of Uptake $\mu\text{g N.g dry wt}^{-1}.\text{h}^{-1}$		
	Nitrate	Nitrite	Ammonium
NO_3	4.970 ± 0.401	-	-
NO_2	-	1.026 ± 0.043	-
NH_4	-	-	1.317 ± 0.144
$\text{NO}_3 + \text{NO}_2$	4.075 ± 0.433	1.011 ± 0.032	-
$\text{NO}_3 + \text{NH}_4$	4.790 ± 0.255	-	1.757 ± 0.135
$\text{NO}_2 + \text{NH}_4$	-	1.058 ± 0.032	1.796 ± 0.156
$\text{NO}_3 + \text{NO}_2 + \text{NH}_4$	5.446 ± 1.451	0.967 ± 0.026	1.648 ± 0.097

reduced in the tissue, darkness must suppress the uptake process itself rather than the reduction of the nitrate taken up. This is considered in more detail in the discussion following.

f Simultaneous Uptake of Nitrate, Nitrite and Ammonium by discs of *L. digitata* over 24 hours in the light

The nitrogen sources were supplied at the following concentrations:

$\text{NO}_3\text{-N}$	7.5 $\mu\text{g-at N.l}^{-1}$
$\text{NO}_2\text{-N}$	1.5 $\mu\text{g-at N.l}^{-1}$
$\text{NH}_4\text{-N}$	3.0 $\mu\text{g-at N.l}^{-1}$

in order to be present in proportion to the concentration found in situ at the time of the experiment (April).

The results (Table 4 vii) show that the rate of nitrate uptake was unaffected by the presence of high concentrations of nitrite and ammonium. The rate of nitrate uptake was not affected by the presence of ammonium only but nitrite, at 1.5 $\mu\text{g-at N.l}^{-1}$ caused a suppression of nitrate uptake ($P < 0.10$). All other combinations were not significant, uptake of nitrite was not affected by high concentrations of nitrate and/or ammonium and similarly uptake rates of ammonium were not affected by high concentrations of nitrate and/or nitrite.

L. digitata, therefore, appears to be able to take up all 3 N forms equally well although nitrate uptake is reduced in the presence of 1.5 $\mu\text{g-at N.l}^{-1}$ nitrite.

reduced in the tissue, darkness must suppress the uptake process itself rather than the reduction of the nitrate taken up. This is considered in more detail in the discussion following.

f Simultaneous Uptake of Nitrate, Nitrite and Ammonium by discs of *L. digitata* over 24 hours in the light

The nitrogen sources were supplied at the following concentrations:

$\text{NO}_3\text{-N}$	$7.5 \mu\text{g-at N.l}^{-1}$
$\text{NO}_2\text{-N}$	$1.5 \mu\text{g-at N.l}^{-1}$
$\text{NH}_4\text{-N}$	$3.0 \mu\text{g-at N.l}^{-1}$

in order to be present in proportion to the concentration found in situ at the time of the experiment (April).

The results (Table 4 vii) show that the rate of nitrate uptake was unaffected by the presence of high concentrations of nitrite and ammonium. The rate of nitrate uptake was not affected by the presence of ammonium only but nitrite, at $1.5 \mu\text{g-at N.l}^{-1}$ caused a suppression of nitrate uptake ($P < 0.10$). All other combinations were not significant, uptake of nitrite was not affected by high concentrations of nitrate and/or ammonium and similarly uptake rates of ammonium were not affected by high concentrations of nitrate and/or nitrite.

L. digitata, therefore, appears to be able to take up all 3 N forms equally well although nitrate uptake is reduced in the presence of $1.5 \mu\text{g-at N.l}^{-1}$ nitrite.

Table 4 viii. Frond dry weight change, % increase in frond surface area and linear growth rate (mean over 20 days) of L. saccharina grown for 20 days at 10 °C, with a photoperiod of 14 hrs L with high phosphate. Nitrogen was supplied at 7.5 $\mu\text{g-at N.l}^{-1}$ and the low-N control contained 0.5 $\mu\text{g-at N.l}^{-1}$. Mean \pm SE, 3 replicates/treatment.

	% inc in frond dry wt	% inc frond surface area	Growth rate mm.day^{-1}
Control	15.00 \pm 3.14	15.33 \pm 4.92	2.37 \pm 0.53
+Urea	26.45 \pm 3.23	24.03 \pm 2.38	3.21 \pm 0.34
+NO ₃	34.03 \pm 4.39	30.44 \pm 5.23	3.53 \pm 0.29
+NO ₂	30.23 \pm 1.25	29.32 \pm 2.73	3.31 \pm 0.23
+NH ₄	30.13 \pm 5.13	23.17 \pm 2.73	3.23 \pm 0.45

Table 4 ix. % change in internal N (total N) content of the meristem and mature tissue of L. saccharina. (See legend to Table 4 viii).

	Meristem	Mature
Control	- 52.85 \pm 3.77	-44.15 \pm 3.78
+Urea	+274.93 \pm 9.35	+78.23 \pm 3.25
+NO ₃	+295.37 \pm 12.66	+35.05 \pm 5.53
+NO ₂	+264.12 \pm 17.65	+30.46 \pm 3.90
+NH ₄	+296.34 \pm 11.29	+87.22 \pm 4.60

- denotes decrease

+ denotes increase

When all 3 forms of nitrogen are present at high concentrations, the rates of uptake of the N sources are in proportions to the levels at which they are supplied; ie. the 3 forms are supplied in the ratio 5:2:1 ($\text{NO}_3:\text{NH}_4:\text{NO}_2$) and the uptake rates are in the ratio 5.6:7.1:1.0.

V GROWTH OF *L. SACCHARINA* ON DIFFERENT NITROGEN SOURCES IN SEPTEMBER

Small whole sporophytes of *L. saccharina* were grown in the laboratory for 20 days at ambient seawater temperature (10°C) and ambient photoperiod (14 hours Light) with high levels of phosphate ($3.0 \mu\text{g-at P.l}^{-1}$). The control treatment contained the ambient nitrogen concentration of the seawater ($0.5 \mu\text{g-at N.l}^{-1}$). In the +Nitrogen treatments, the different nitrogen sources were supplied at $7.5 \mu\text{g-at N.l}^{-1}$.

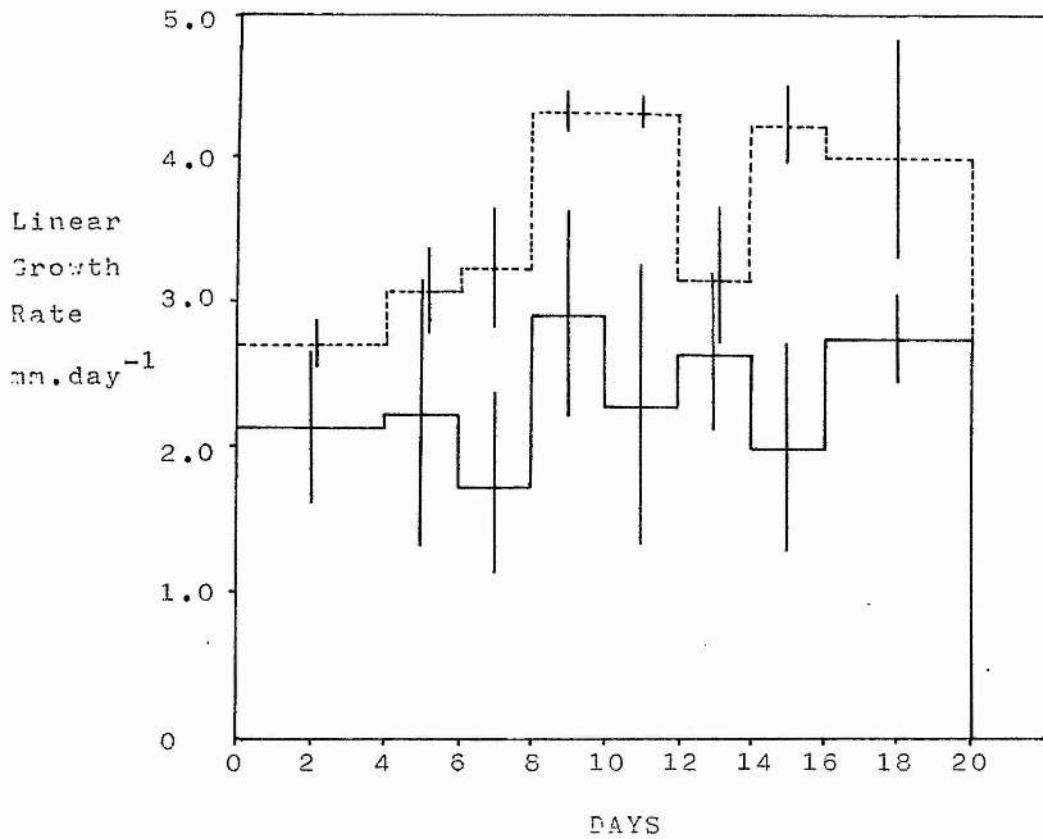
The fronds increased in dry weight over the course of 20 days in all treatments (Table 4 viii) but this increase was only significantly greater than the low-N control when nitrate or nitrite was added. The mean linear growth rate over 20 days (Table 4 viii) was similar between all treatments and the frond surface area only increased significantly above that of the control when nitrate was supplied at $7.5 \mu\text{g-at N.l}^{-1}$.

The change in total internal N content of the meristem and mature tissue over the course of the experiment is shown in Table 4 ix. In the controls the total N content of both tissue areas is reduced significantly over 20 days,

Fig. 4 xxv. Linear growth rate of the frond (mm.day^{-1}) of *L. saccharina* grown in low-N seawater ($0.5 \mu\text{g-at N.l}^{-1}$. Control) and with added nitrate ($7.5 \mu\text{g-at N.l}^{-1}$) over the course of 20 days in September.

(Mean \pm SE. 3 replicates/treatment)

----- +Nitrate
 — Control



The total N content was significantly higher than the controls in all +N treatments ($P < 0.001$) but the difference between all +N treatments was not significant.

The low-N controls appear, therefore, to be utilising internal N reserves to maintain growth rates similar to the +N treatment growth rates. Over the course of the experiment linear growth rate remains relatively constant in the controls (Fig. 4 xxv) but if the experiment had been extended in time, it is suggested that growth rates would decline as internal reserves became further depleted. With supplementary N, the mean linear growth rate is not raised significantly above the low-N controls. During the experiment the linear growth rate of +Nitrate sporophytes shows an initial increase 0-8 days but levels off after this (Fig. 4 xxv) although the tissue continues to accumulate N, and the final growth rate (16-20 days) is not significantly higher than during 0-4 days. Some factor must become limiting to growth and it is unlikely to be phosphate concentration, photoperiod, seawater temperature or nitrogen concentration which are all high at this time. It is possible that the tissue has become senescent and has only a very limited response to increased nitrate. In situ tissue-N content is largely determined by seawater N concentration (4 iii a), however in this experiment the tissue N content of the nitrate enriched treatment is lower than predicted from the external concentration;

External Conc. of control and + nitrate treatment is

in the ratio 1.0 : 15.0.

Tissue-N content of the control and the +nitrate treatment is in the ratio 1.0 : 8.4.

The ratio of the mean linear growth rate between the control and +nitrate treatment ($\frac{3.58}{2.37} = 1.51$) is considerably lower than predicted from either the seawater concentration or the tissue-N content. L. saccharina is, therefore, not responding in September in the same way to increased or high N as it does in the Spring (4 iii c) suggesting some factor(s) limiting growth. As it is unlikely to be phosphate or nitrate concentration, seawater temperature or photoperiod, senescence is proposed as a possible factor limiting Laminaria response to apparently 'ideal' conditions.

DISCUSSION

The typical seasonal fluctuation of nitrate and nitrite in the seawater is well known but in contrast to some locations (English Channel; Harvey, 1926) nitrate is never completely exhausted at the sampling sites as agricultural run-off and pollution continuously replenish supplies. Ammonium remains relatively high during the summer but concentrations approximate to minimum summer levels of nitrate and it is the variation in nitrate which, therefore, determines the overall seasonal fluctuation in seawater nitrogen. The tissue-N content of L. saccharina and L. digitata follows the seawater N concentration and is high in the winter and low during the summer.

Laminaria spp. accumulate nitrogen in excess to growth requirements during the winter, indicated by the increasing internal organic and inorganic-N reaching a maximum content per unit dry weight in April. With adequate N (both externally and internally) much of the nitrate taken up is retained (and is extractable in short-term uptake experiments) as nitrate (inorganic-N). Since the organic-N content also increases during the winter, a proportion of the inorganic-N taken up must be used for reduction and amination and there is also some storage of N as non-protein and perhaps some protein N. The large remaining pool of inorganic-N may, therefore, represent a biophysical surplus to metabolic requirements.

The decline in seawater N in April occurs as accumulation of N by Laminaria and other macroalgae (eg. Ascophyllum nodosum (Black, 1948); Fucus spp. (Black, 1949)) reaches a maximum and phytoplankton density peaks at St. Andrews (Richardson, 1969). Total internal N drops sharply as growth rates continue to increase for a further month; inorganic-N is utilised most rapidly followed by organic (non-protein)-N as the conversion to protein and dilution by other algal constituents continues. The low external N and the depletion of tissue NO_3 restricts the net synthesis of further low molecular weight organic-N compounds which, in turn, limits protein synthesis. This restriction on protein synthesis resulting from the reduced availability of N (both internally and externally) may cause a decrease in linear growth rates after a lag period of one month; this

must be the time required for utilisation of stored reserves. This decline in growth, in turn, limits the utilisation of photosynthate for growth at a time of maximum production, hence mannitol and subsequently laminarin accumulate. In the meristem tissue-N declines during the summer as it is diluted by other cellular components eg. carbohydrates, and cell division and displacement of tissue distally removes N from the meristem as external N limitation prevents rapid uptake and accumulation. In the mature frond tissue where growth (cell division and cell enlargement) has ceased the N content also declines. The dry weight of the frond increases during the summer (as carbohydrates accumulate) and the decline in tissue-N relative to the dry weight may merely reflect this increase. Using data for L. digitata (mature tissue), the mean dry wt. of a disc of tissue (2.5 cm diameter) increases from 42.94 ± 1.34 mg in April to 71.09 ± 3.22 mg by September; the actual amount of N (μg) in that area of tissue drops from 1339.39 to 514.95 μg . In April this N content represents 3.06% of the dry wt. If the amount of N remains constant then it would comprise only 1.92% of the dry wt. in September. The observed N content (0.84% dry wt.) is less than the predicted value and the decline in N therefore, represents an actual loss of N from that area of tissue.

The N may be lost in three ways:

- i Loss to the seawater (efflux)
- ii Losses by respiration
- iii Basipetal translocation

Despite the availability of ^{15}N no bidirectional flux measurements of NO_3^- (or NH_4^+) appear to have been made in algae (Raven, 1975), possibly due to the experimental difficulties resulting from the requirement for aseptic conditions to eliminate surface microbial effects. Evidence for other anions eg. PO_4^- and SO_4^{2-} suggests that efflux is low relative to unidirectional influx (which approximates to the measured net influx) (Vallee & Jeanjean, 1968; Raven, 1974). Loss of nitrogenous materials during adverse conditions is reported by Sieburth (1969) but this is unlikely to be important in midsummer. Unicellular algae are reported to release proteins, peptides and amino acids into the seawater under normal conditions (Khailov & Burlakova, 1969) but there is no evidence for this in macroalgae. Loss of N by these means is, therefore, probably low.

It has been suggested that amino acids rather than mannitol are utilised as the principle respiratory substrate in L. digitata (Hellebust & Haug, 1972b); however, this would only result in a drop in tissue-N content if their N is subsequently exported. Quantitative measurements of utilisation of amino acids and subsequent export (if it occurs) have not been attempted.

Translocation of amino acids from mature to meristematic tissue has been shown to occur in the Laminariales, but despite extensive studies on the rates of translocation and composition of the translocate, there is little information on the actual amounts of N exported. All measurements are relative to the amount of labelling eg. amino acids comprised

32% of the total basipetally exported ^{14}C -labelled translocate in L. saccharina; 71% of the total basipetally exported translocate accumulated in the lowermost 20 cm of the frond in 96 hours (Luning et al, 1973) and actual amounts ($\mu\text{g N}$) exported over the course of the experiment are not estimated. It is, therefore, not possible to establish whether the loss of N from the distal frond tissue (824 μg from April to September) may be accounted for by basipetal translocation; but since efflux is presumed to be low, N must, by inference, be exported from the mature frond tissue either by basipetal translocation of amino acids or N-products from respiratory breakdown of amino acids.

To summarise: evidence presented suggests that during the winter 'luxury consumption' of N occurs by Laminaria spp; the stored N reserves are utilised to maintain maximum growth rates after N in the seawater has declined. However, prolonged external limitation causes a decline in linear growth rates, by restricting protein synthesis, once internal N reserves have been depleted, despite the probable translocation of N from the mature to the meristematic frond tissue.

Seawater N concentration and tissue N content are positively correlated and in areas where the seawater N is high (as a result of pollution) the internal total N content is also proportionally higher. However, growth rates are not directly determined by total available external N concentration since a small rise in external N results in a greatly increased growth rate. High levels of all 3 N forms

$(\text{NO}_3 + \text{NO}_2 + \text{NH}_4)$ does not explain the higher growth rates and similarly, the higher phosphate concentration in addition to the N at the Sewer site predicts a lower growth rate than is observed. Plants at St. Andrews Sewer site are growing at extreme low water of spring tides (ELWS) and are therefore, immersed for considerably longer periods than plants higher up the shore (ie. at St. Andrews site and also at Kingsbarns and Fifeness), and during parts of the winter (from December until February or March) they are only emersed for short periods during each tidal cycle or sometimes not at all. As a result, there is an almost continuous movement of water past the frond surface (except when emersed) which effectively reduces the width of the boundary layer (Wheeler, 1980) and enhances nutrient uptake (Whitford & Schumacher, 1961; Doty, 1971; Keushal, 1972). Nutrient uptake of plants higher up the shore during periods of emersion is restricted to the diffusion of ions retained in the film of moisture on the frond surface. Following from this it would be expected that Laminaria growing in pools isolated at low water would also have higher growth rates than plants which are emersed. This was not found to occur at St. Andrews and may result from only limited water movement in the pools during low tide and, therefore, similar diffusive limitations on nutrient uptake. It is proposed that the high growth rates of plants at the Sewer site result from increased periods of water movement past the frond surface in conjunction with the elevated N ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4$) and phosphate concentrations found in this locality.

L. digitata has been shown to take up nitrate, nitrite and ammonium simultaneously from the medium; this confers a competitive advantage over other macroalgae and phytoplankton many of which show either a preference for one N form or suppression of uptake of one form of N in the presence of another. This advantage is particularly important in the summer when external N concentration is minimal. Simultaneous uptake occurs in a number of algae, where uptake of one is not affected by the other forms: in Gelidium nudifrons (Bird, 1976), Fucus spiralis (Topinka, 1978), Macrocystis pyrifera (Haines & Wheeler, 1977), L. longicruris (Harlin & Craigie, 1978). In other species ammonium causes suppression of nitrate uptake: in Hypnea musciformis NO_3^- uptake over 80 minutes was reduced by half in the presence of equimolar NH_4^+-N , but NO_3^- had no effect on NH_4^+ uptake (Haines & Wheeler, 1978) and in Gracilaria foliifera and Neogardhiella baileyi NO_3^- uptake was suppressed at $5 \mu\text{M}(\text{NH}_4^+)$ but simultaneous uptake occurs at unsuppressed rates at lower concentrations (D'Elia & DeBoer, 1978).

In L. digitata, NO_3^- uptake was suppressed in the presence of NO_2^- only but NO_2^- was unaffected by high concentrations of NO_3^- . In L. longicruris, Harlin & Craigie (1978) found a similar suppression of nitrate as the molar ratio of $\text{NO}_2^-:\text{NO}_3^-$ increased, and in Codium fragile (Hanisak, 1979b) uptake of NO_3^- was unaffected by the presence of NO_2^- , but NO_2^- uptake was reduced by half when $\text{NO}_3^-:\text{NO}_2^-$ was in the ratio $5 \mu\text{M}:5 \mu\text{M}$. However, comparison between individual studies is difficult; uptake is frequently measured at concentrations

higher than would normally be found in situ and the physiological state of the experimental plants (whether N limited prior to the experiment and the levels of internal N reserves) are not always stated.

Uptake of nitrate by L. saccharina and L. digitata was very similar despite the use of whole plants of L. saccharina and discs cut from the meristem of L. digitata, emphasising the validity of using tissue discs in nutrient uptake experiments.

The 2 phase uptake of nitrate has been previously observed in corn (Van Den Honert & Hooymans, 1955) and in Macrocystis, ammonium shows a similar biphasic uptake (Haines & Wheeler, 1978). The mechanism to explain this biphasic uptake (at present the multiphasic model (Nissen, 1973) takes precedence over the parallel (Epstein, 1972) and the series (Laties, 1969) models) need not concern us here. Uptake of nitrate by Laminaria is saturated at nitrate concentrations which are higher than is normally found in situ during the winter (ie. $25-30 \mu\text{g-at N.l}^{-1}$); however, the excess of uptake capacity enables Laminaria to take advantage of any highly concentrated localised pockets of nitrate which may be brought close to the frond surface. Nitrate can be taken up efficiently from very low external concentrations; K_m is low or site affinity is high. Uptake during the summer must, therefore, be limited by supply of nitrate to the frond surface, particularly critical when turbulence is at a minimum and seawater N concentrations are low.

Nitrate uptake was reduced in the dark in L. digitata in agreement with other workers, eg. in L. longicruris (Harlin & Craigie, 1978). Since little of the nitrate taken up is reduced in the tissue, the uptake process rather than the reduction of the nitrate taken up must be suppressed. Whatever the mechanism of NO_3^- influx, darkening would decrease the rate of regeneration of ATP (through phosphorylation), which might be the ultimate or the proximate energy source for continued active NO_3^- influx. Since intracellular free NO_3^- levels approach those externally, and Laminaria cells are likely to be electrically negative to the medium, then nitrate uptake is almost certain (on electrochemical grounds) to be an active transport process dependent on continued energy supply eg of ATP. These experiments were short (24 hours), as a result, decreased uptake in darkness is less likely to be caused by loss of uptake site capacity than to be a kinetic effect of reduced energy supply for existing transport sites.

Since uptake is limited by supply to the frond surface and, during the summer the external N concentration rather than light (daylength) is the main limitation on uptake, depression of nitrate uptake in the dark is probably not very relevant to Laminaria growth in situ. Depression of nitrate uptake in the dark is probably not significant in the winter either since uptake is maximal despite short days. This is unlikely to be an actual increase in uptake rate per unit area, but the rate of uptake remaining the same; the high external N concentration no longer limits

supply to the frond surface.

L. saccharina can grow on and take up a number of different nitrogen sources but growth rates in September were not significantly greater than those of plants utilising stored reserves (the low-N controls). Some factor(s) become limiting to growth after 8 days and growth rates of +N treatments were not as great as predicted from the internal N content. Light (photoperiod and irradiance), temperature, phosphate and nitrate concentration are unlikely to be limiting to growth at this time. It is suggested (and expanded further in Chapter 6; Interaction of N and P) that by September the frond tissue may have undergone senescence (either endogenously or exogenously induced). The tissue loses, to a large extent, its ability for cell division and cell enlargement and as a result there is only a limited response to increasing external N concentration; the ability for uptake and accumulation of N is retained as is consistent with a change in cell fate or senescence.

In situ, Laminaria shows a small transient growth increase in September as N (and P) increase in the seawater. This growth increase may represent the extent of the response possible by Laminaria to the increased external and subsequently increased internal N levels. Whether this senescence effect is exogenously (possibly nutrient) or endogenously induced is discussed in Chapter 6.

To conclude: Laminaria spp. accumulate N in excess to immediate metabolic requirements during the winter; the N

reserves, stored mainly as inorganic-N but also as organic (non-protein)-N and perhaps some protein, are utilised to maintain maximum growth rates after N in the seawater has declined in the spring. Prolonged external limitation causes a decline in growth rates by restricting protein synthesis once internal N reserves have been depleted and senescence of the tissue by September prevents a rapid increase in growth rate as seawater N increases. The tissue N content is largely determined by the external N concentration (NO_3 , NO_2 and NH_4 , since Laminaria spp. can take up all 3 forms simultaneously) but growth rates are only indirectly determined by internal and external N concentration. Water movement passed the frond surface to reduce the thickness of the boundary layer and to continuously replenish nutrients is particularly important in this context as uptake of nitrate by L. digitata and L. saccharina is limited by supply of ions to the frond surface.

CHAPTER 5

PHOSPHATE AND GROWTH

INTRODUCTION

The seasonal fluctuation of phosphate in the sea has been known for some time (eg Cooper, 1938) and the relationship between phosphate concentration and phytoplankton biomass has been extensively investigated (Harvey, 1940; Ryther & Dunstan, 1971; Gerhart & Likens, 1975). The effect of seasonal fluctuations in phosphate on the growth of marine macroalgae has been relatively little investigated and correlations between external $\text{PO}_4\text{-P}$ concentration and internal P levels has not previously been attempted.

From phytoplankton studies Ryther & Dunstan (1971) found that in coastal marine water phosphate is generally in excess to growth requirements (often 2 x as much is present as can be utilised) and they concluded that nitrogen is the critical limiting factor to micro-algal growth. Gargas (1975) from theoretical calculations similarly concluded that phosphorus cannot possibly have been a growth limiting factor, in a Danish fjord, nitrogen was much more important.

From work on macroalgae it also appears that nitrogen rather than phosphate is a more important factor likely to limit growth. Chapman & Craigie (1977) showed the importance of N in influencing the seasonal growth pattern of L. longicruris, in an area where phosphate

was not depleted during the summer to very low levels. However, in a location where phosphate becomes depleted during the summer it is possible that it too may be an important limitation on growth. In Alaria esculenta, Buggeln (1974) found enhanced summer growth with the addition of both NO_3 and PO_4 , suggesting both N and P limitation in situ, but he did not attempt to investigate the effect on growth by the addition of N and P separately.

The effect of the seasonal fluctuations in exogenous PO_4 -P on the seasonal growth of L. digitata and L. saccharina is examined to investigate to what extent growth of Laminaria is influenced by seawater phosphate concentration and whether P is limiting to growth during the summer months. This is estimated using correlations of seawater PO_4 -P concentrations with internal levels of P and with growth rates and in addition, uptake of phosphate and growth of Laminaria in P-enriched seawater during the late summer (September) is investigated.

RESULTS

i EXOGENOUS PO_4 -P

a Seasonal variation of phosphate in the sea

PO_4 -P in the sea follows the typical seasonal pattern of North Temperate waters, and is similar to the seasonal fluctuation of NO_3 -N (Chapter 4). PO_4 -P declines very rapidly in the sea in April to low levels throughout the summer and then begins to increase in

Fig. 5 i. Seawater phosphate concentration during 1979-1980 at St. Andrews.

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Fig. 5 ii. Seawater $\text{PO}_4\text{-P}$ concentration during 1979-1980 at Kingsbarns.

Fig. 5 iii. Seawater $\text{PO}_4\text{-P}$ concentration during 1979-1980 at Fifeness.

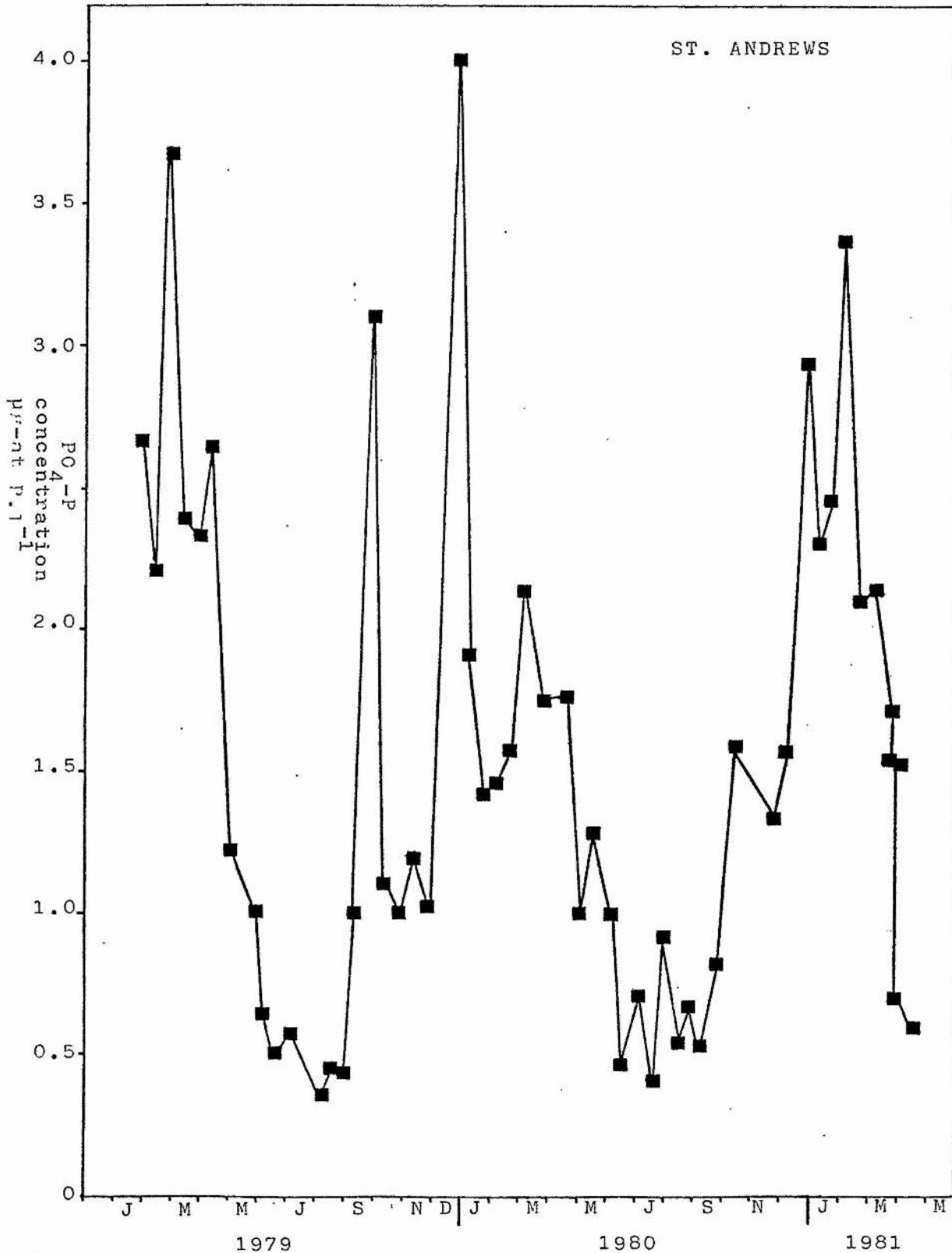


Fig. 5 ii.

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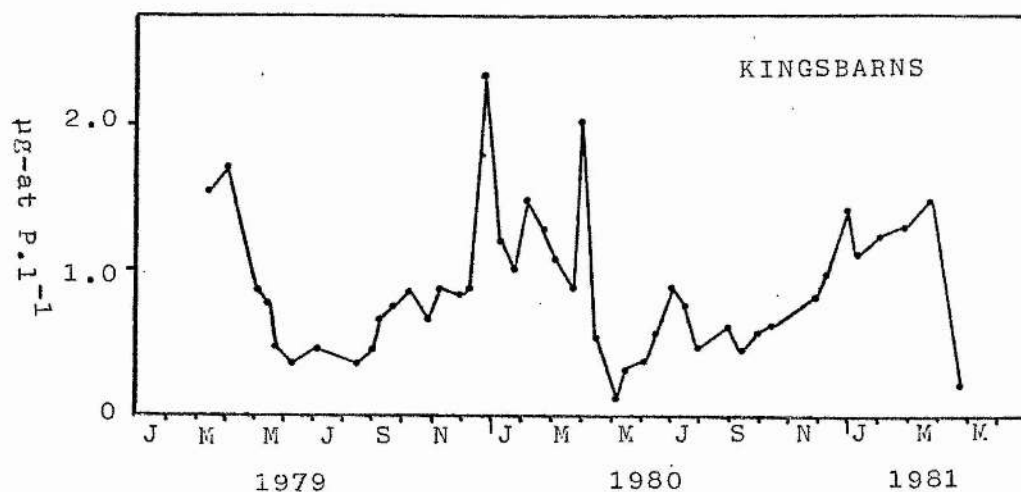


Fig. 5 iii.

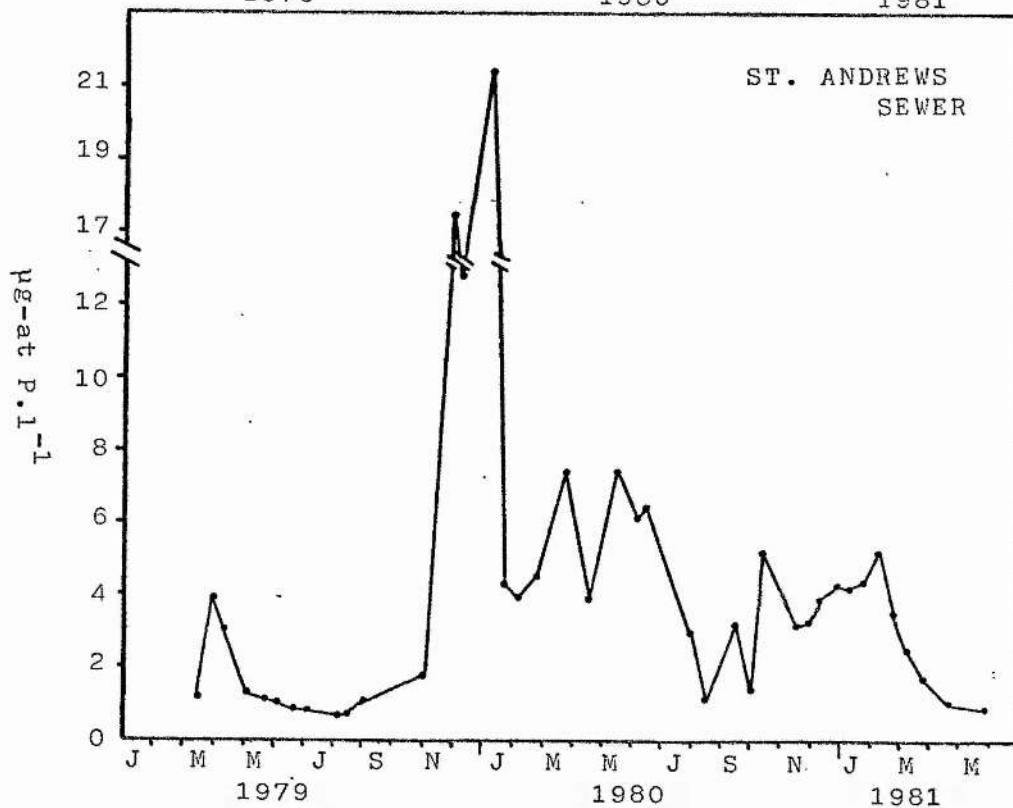
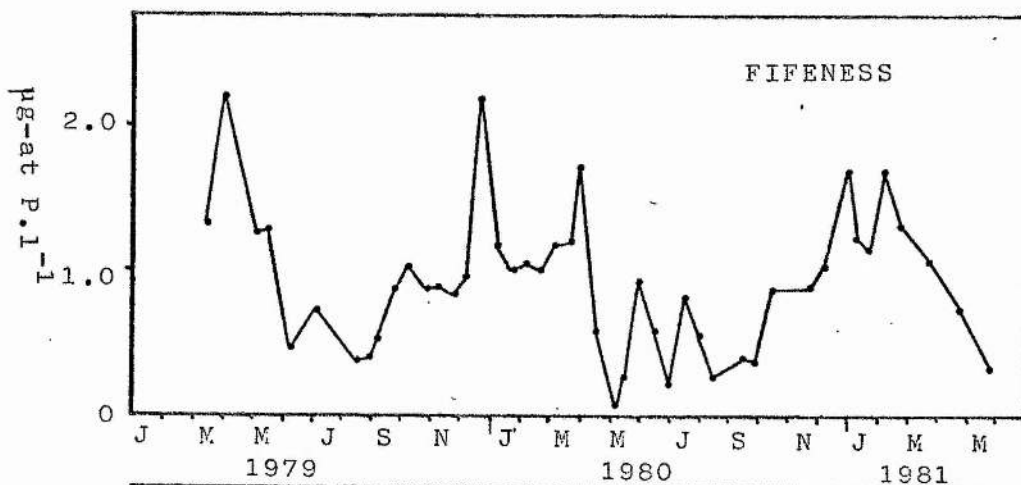


Fig. 5 iv. Seawater PO₄-P concentration during 1979-1981 at St. Andrews Sewer.

late summer (September) to maximum concentrations during the winter months (Figs 5 i-iv).

b Variation in seawater phosphate concentration between sites

This seasonal pattern in $\text{PO}_4\text{-P}$ concentration is exhibited at all 4 sampling sites. $\text{PO}_4\text{-P}$ levels are not significantly different between Kingsbarns and Fifeness as indicated by the similarity in summer minimum levels at the 2 sites and also similar winter maxima (Table 5 i).

Table 5 i. The mean summer and winter seawater $\text{PO}_4\text{-P}$ concentration at the 4 sites. (Mean \pm SE)

SITE	SUMMER 1980 $\mu\text{g-at P/l}$	WINTER 1980/1981 $\mu\text{g-at P/l}$
Kingsbarns	0.513 \pm 0.062	1.115 \pm 0.105
Fifeness	0.446 \pm 0.076	1.212 \pm 0.103
St. Andrews	0.736 \pm 0.086	1.884 \pm 0.208
Sewer	4.099 \pm 0.948	3.541 \pm 0.409

Phosphate concentration at St. Andrews is significantly higher than at Kingsbarns and Fifeness during the summer ($P < 0.05$) and the winter ($P < 0.01$), but water from the sewer outlet has significantly more phosphate than the other 3 sites during the winter (Kingsbarns, $P < 0.001$; Fifeness, $P < 0.001$; St. Andrews, $P < 0.002$) and the summer months ($P < 0.001$; $P < 0.001$; and $P < 0.001$; at the 3 sites

respectively).

The mean summer concentration at the Sewer is higher than the mean winter concentration as a result of 3 high values in May and June 1980. These spurious results may be due to heavy rain preceding the sampling dates leading to a greater discharge of effluent with high PO_4 -P. In 1979 and 1981 the PO_4 -P concentration dropped markedly in May/June without these wide fluctuations.

ii INTERNAL P

a Seasonal Variation

L. saccharina and L. digitata show a similar seasonal variation. Internal P is at maximum levels during the winter months (December to February/March), dropping rapidly during April to low levels during May - June. P content increases to a secondary peak in August, but falls during September before rising to the winter maximum (Figs 5 v-xii). The rapid drop in internal P coincides with the rapid decline in external P in April, while the increase in external P after September is seen with a corresponding increase in internal P.

b Variation in internal P content between distal and meristematic frond tissue

In L. saccharina the maximum relative P content of the meristem is much lower than the maximum P content of the distal frond tissue, but the minimum values are not significantly different, although the meristem content is often higher than that of the older tissue during the summer. The meristem content tends to follow changes in

Fig. 5 v. Tissue P content of the meristem and mature frond tissue of *L. saccharina* during 1980 at St. Andrews.

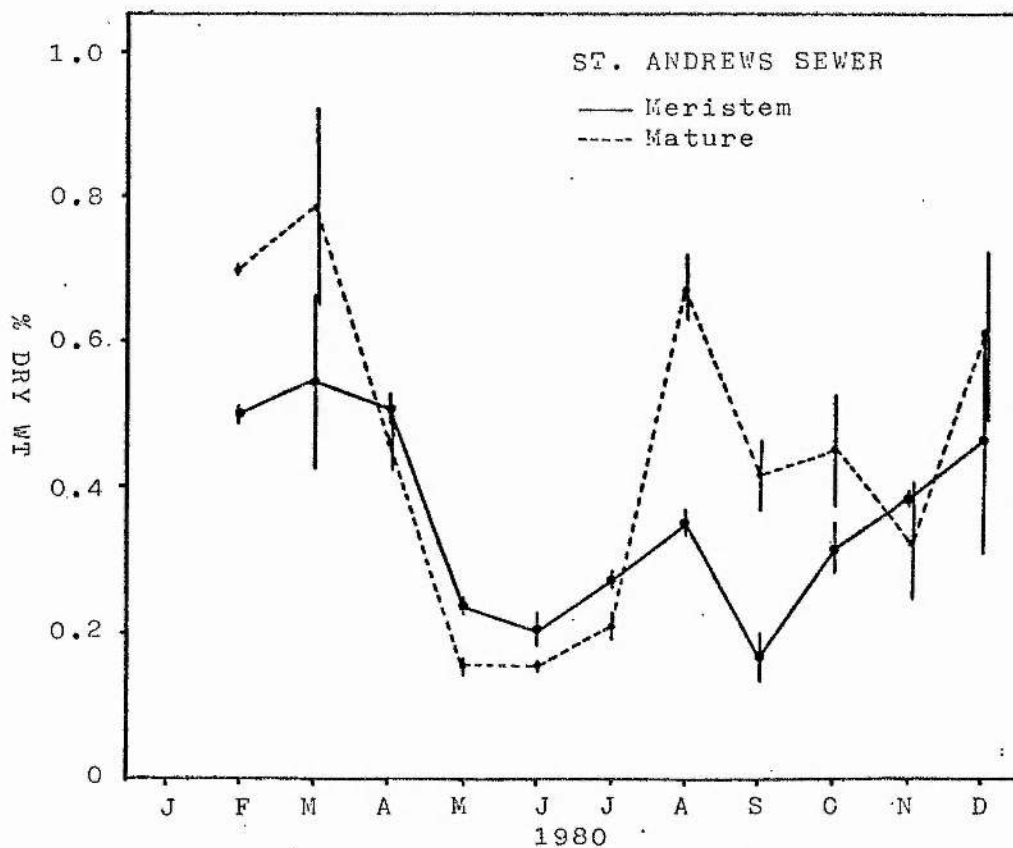
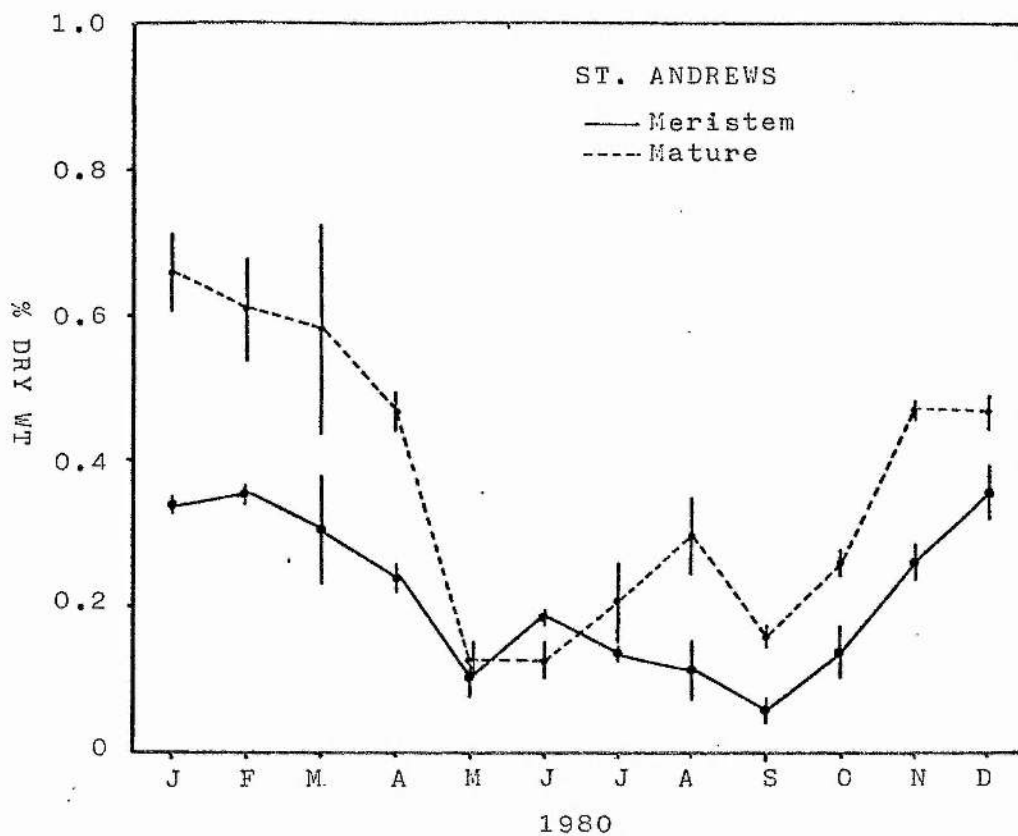


Fig. 5 vi. Tissue P content of the meristem and mature frond tissue of *L. saccharina* during 1980 at St. Andrews Sewer.

Fig. 5 vii. Tissue P content of the meristem and mature frond tissue of *L. saccharina* during 1980 at Fifeness.

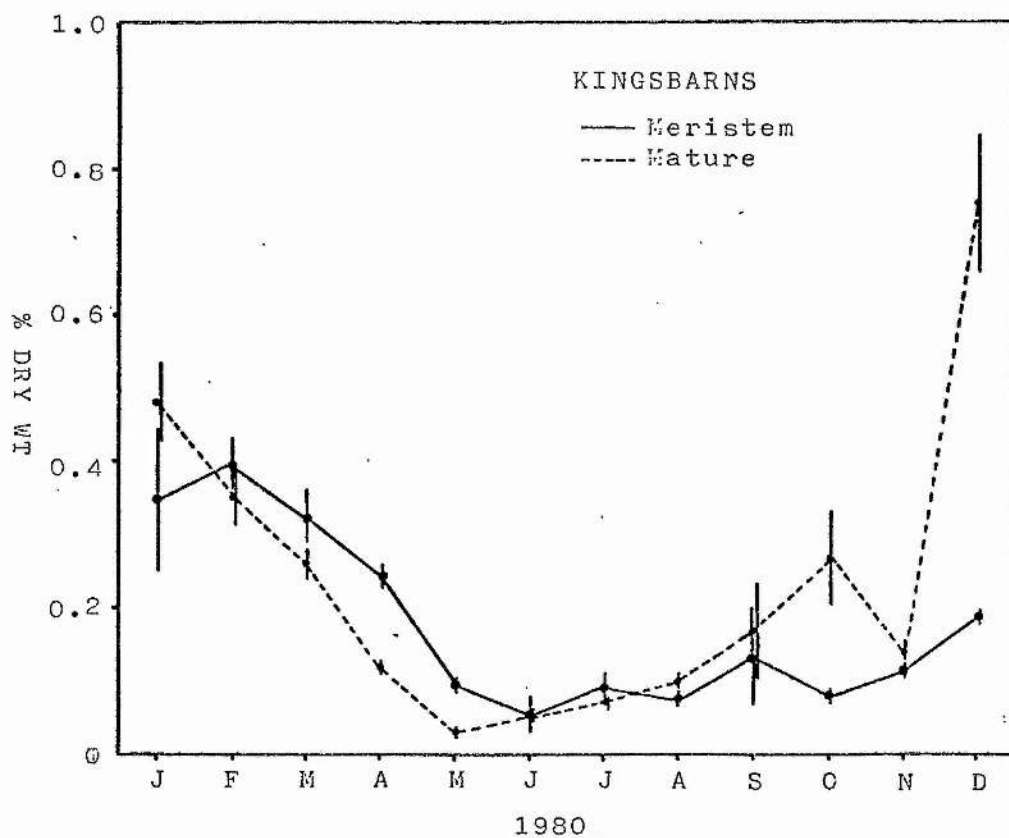
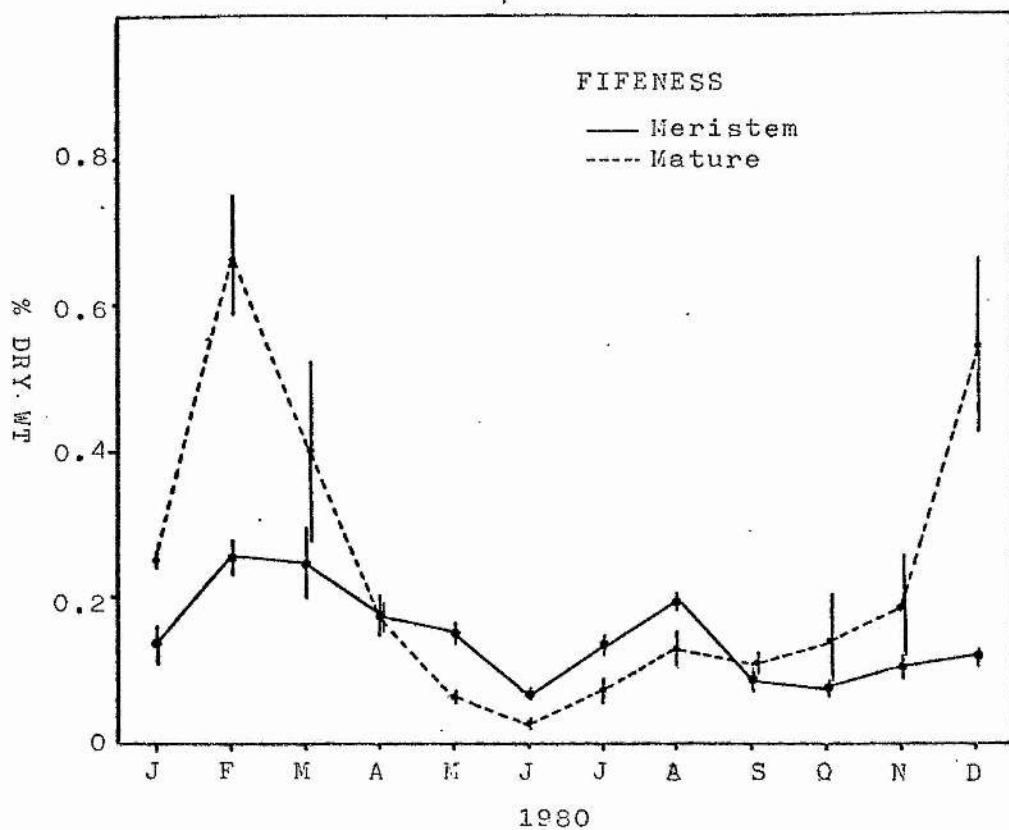


Fig. 5 viii. Tissue P content of the meristem and mature frond tissue of *L. saccharina* during 1980 at Kingsbarns.

Fig. 5 ix. Tissue P content of the meristem and mature frond tissue of *L. digitata* during 1980 at St. Andrews.

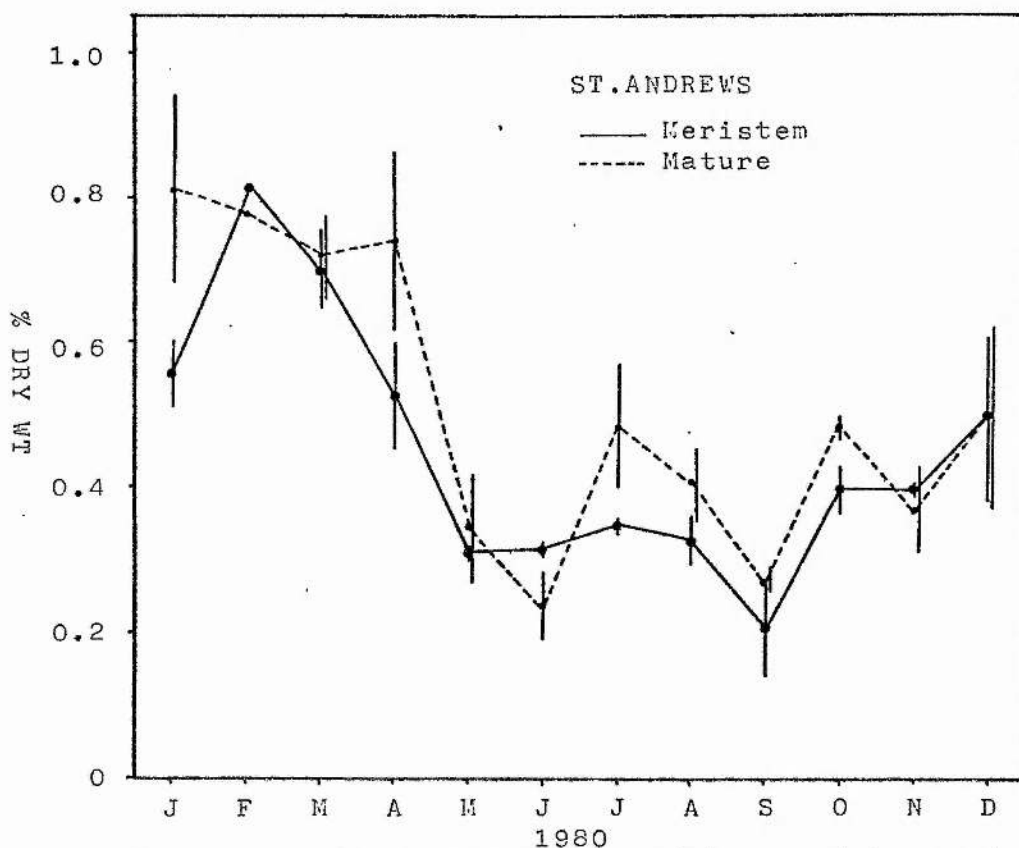


Fig. 5 x. Tissue P content of the meristem and mature frond tissue of *L. digitata* during 1980 at St. Andrews Sewer.

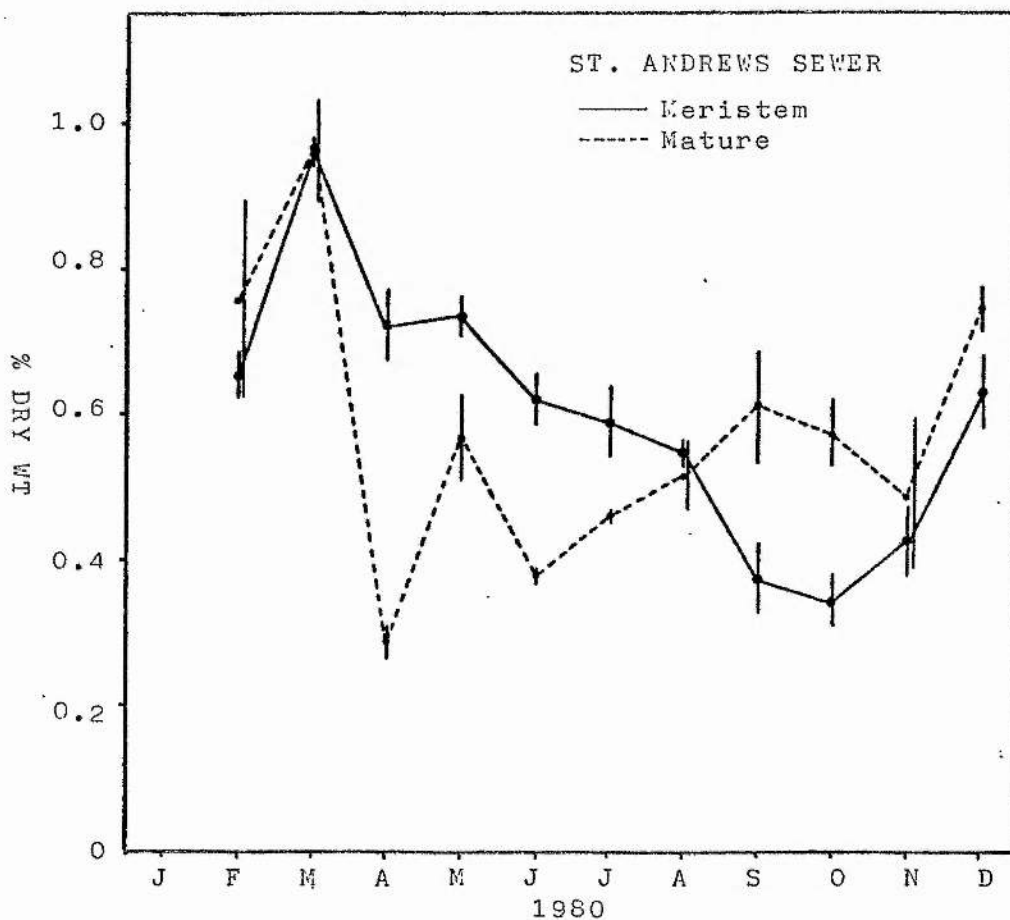


Fig. 5 xi. Tissue P content of the meristem and mature frond tissue of L. digitata during 1980 at Fifeness.

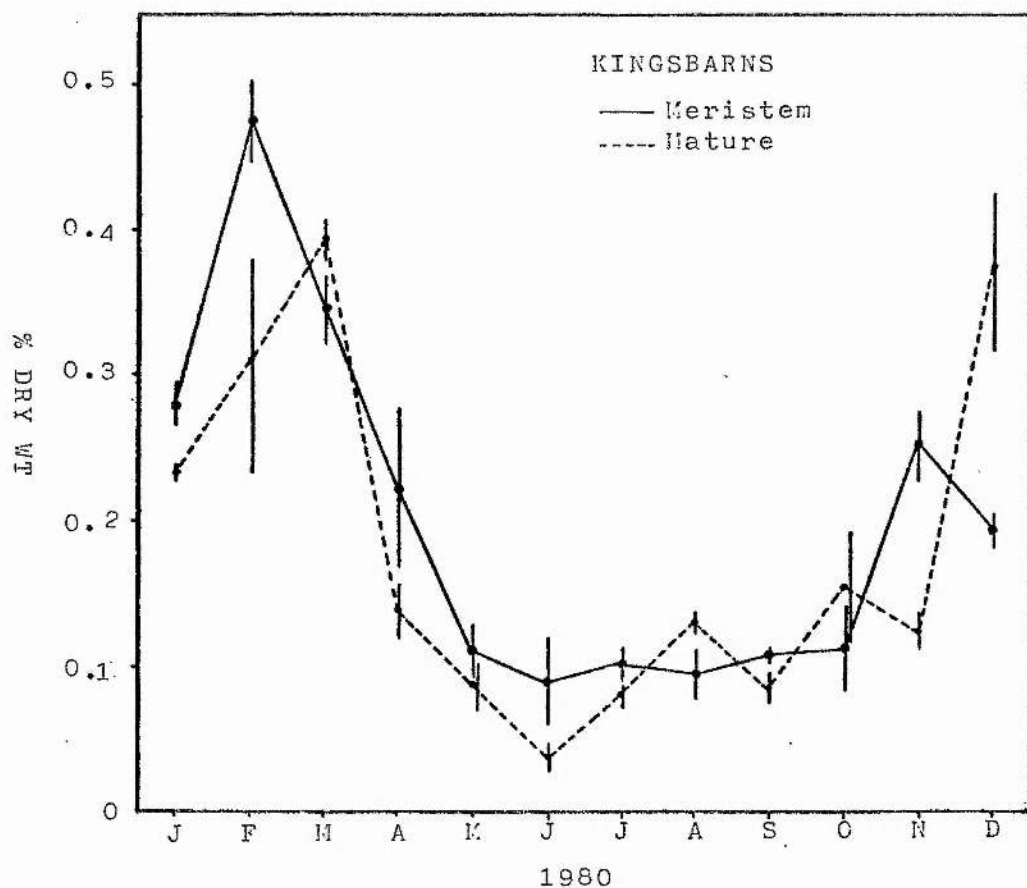
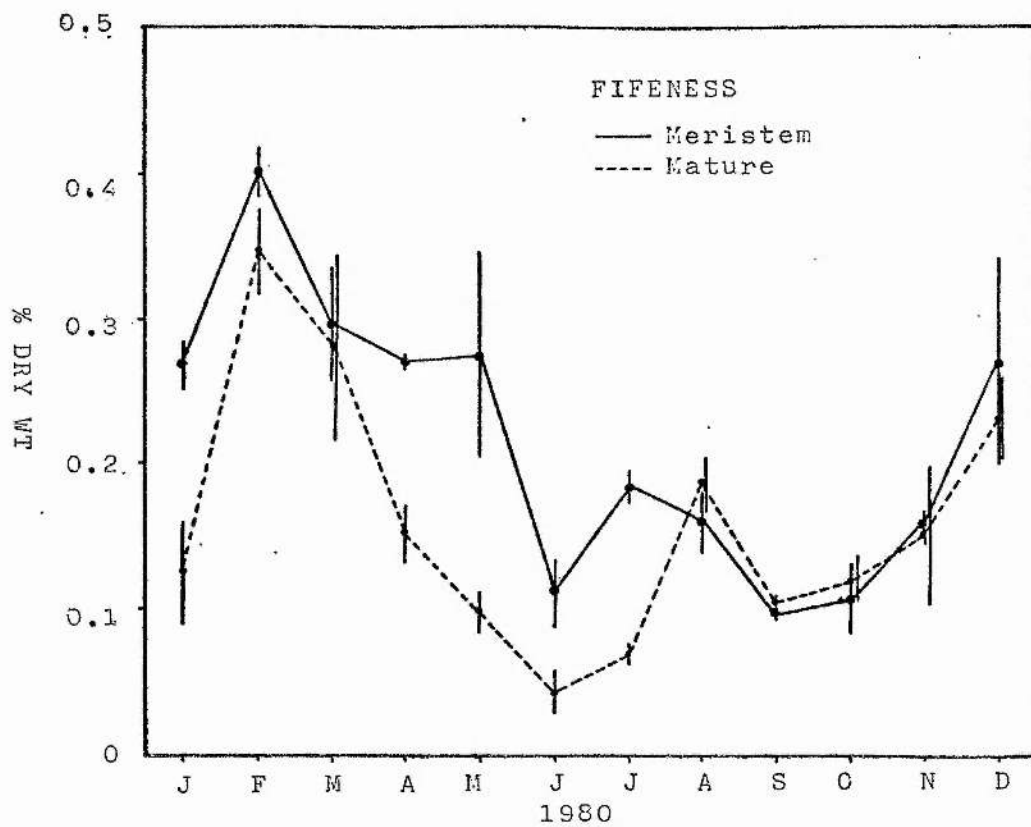


Fig. 5 xii. Tissue P content of the meristem and mature frond tissue of L. digitata during 1980 at Kingsbarns.

distal tissue content by one month i.e. at Sewer, the distal tissue content begins to decline rapidly in March whereas the rapid decline in the meristem begins in April.

In L. digitata, however, it is not apparent that distal tissue fluctuations precede those of the meristem and unlike L. saccharina maximum values are similar in both frond areas. Minimum values are also not significantly different.

Tissue age and physiological state (that is, actively growing or non-actively growing tissue) appears to have little effect on internal P content.

c Variation in internal P content between sites

In the meristem of L. saccharina the maximum P content was similar at St. Andrews, Sewer and Kingsbarns but Fifeness was significantly lower than the 3 other sites ($P < 0.05$). L. saccharina from Sewer had a higher minimum P content than the other sites ($P < 0.05$) which were similar in their minimum levels.

In the distal frond tissue of L. saccharina the maximum P content was similar at all 4 sites but Kingsbarns and Fifeness had significantly less P at the minimum content than both Sewer ($P < 0.001$) and St. Andrews ($P < 0.05$). In the distal tissue, therefore, the P content of plants from the enriched sites (St. Andrews and St. Andrews Sewer) did not drop to such low levels during the summer as those from the relatively unpolluted sites. However, the maximum P content is unaffected by the different external phosphate concentrations. There is no obvious

explanation as to why the minimum P content of the meristem at Fifeness is so much lower than elsewhere during the summer.

In L. digitata, the minimum P content of the meristem is greater at Sewer than at the other 3 sites (St. Andrews, NS; Fifeness, $P < 0.02$; Kingsbarns, $P < 0.10$), and the maximum P content is significantly higher at the Sewer and Kingsbarns than at St. Andrews ($P < 0.05$) and Fifeness ($P < 0.05$). In the distal frond tissue the enriched sites (St. Andrews and Sewer) have a significantly higher ($P < 0.01$) minimum P content than Kingsbarns and Fifeness, and Sewer is higher than St. Andrews (NS). Kingsbarns ($P < 0.10$) and Fifeness ($P < 0.05$) at the maximum P content.

Therefore, at the 2 enriched sites, external PO_4-P levels during the summer are less limiting to L. digitata than at the low external phosphate sites, indicated by a higher minimum P content at St. Andrews Sewer and at St. Andrews than at Fifeness and Kingsbarns, in both the meristem and the older tissue.

iii GROWTH AND EXOGENOUS PO_4-P CONCENTRATION

Using data from St. Andrews (Fig. 5 xiii).

In both L. saccharina and L. digitata from January - April, growth rate increases as exogenous PO_4-P is at maximum concentrations, and from May - August/September growth rates decline as seawater PO_4-P drops to minimum levels. In both species, the rapid decline in growth rates occurs after June as seawater phosphate falls below

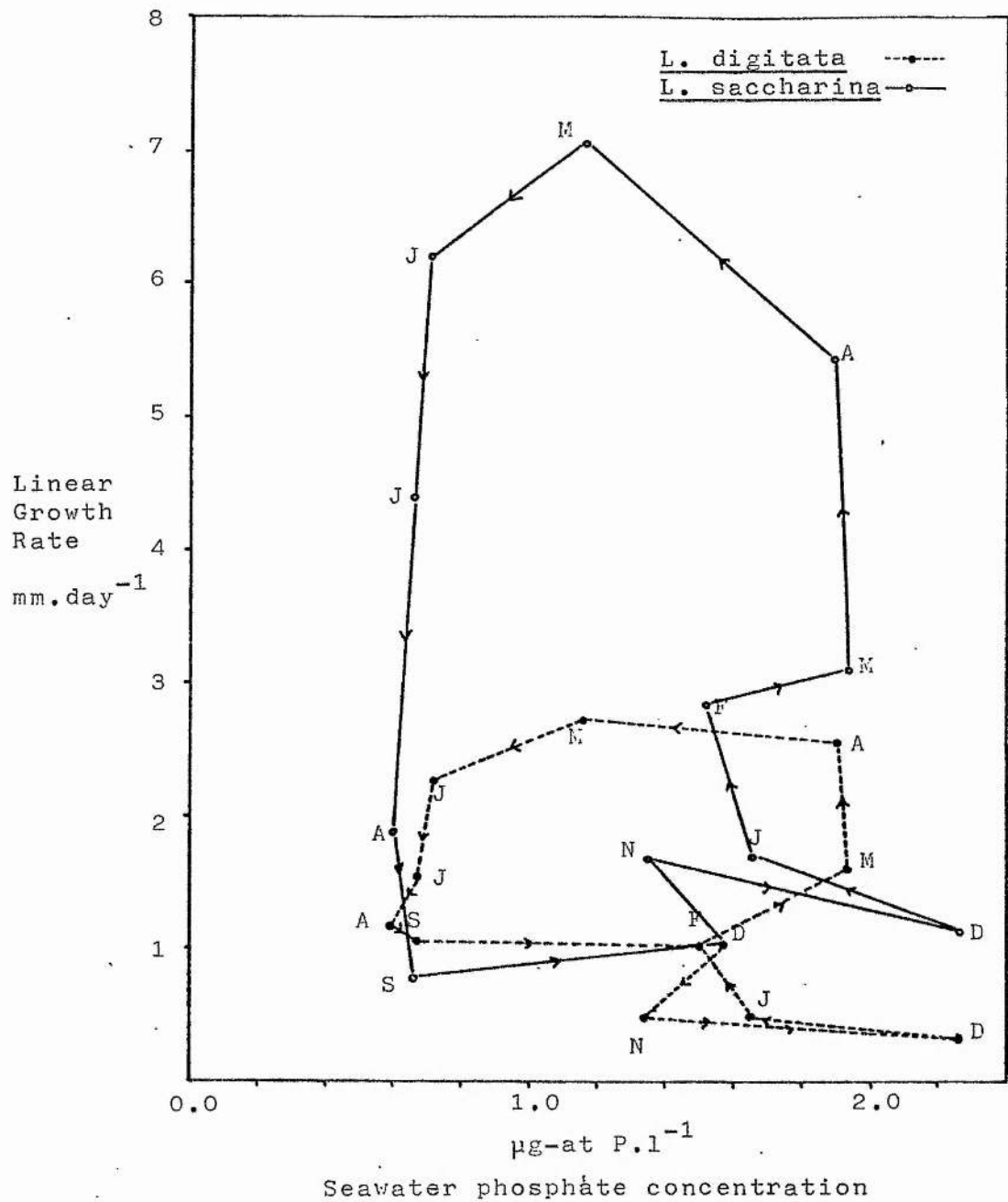


Fig. 5 xiii. Relationship between external phosphate concentration and growth of *L. saccharina* and *L. digitata* during 1980.

0.72 $\mu\text{g-at P/l.}$ L. saccharina shows a significant positive linear correlation ($P < 0.01$) between growth rate and exogenous phosphate concentration during June to August and L. digitata also exhibits a positive linear correlation at this time. This correlation may suggest a causal relationship, with growth rates declining as a result of decreasing external $\text{PO}_4\text{-P}$ concentrations.

L. saccharina shows a slight increase in growth rates in September/October as seawater phosphate increases. This may be a direct response to increasing exogenous phosphate although nitrate, which is also increasing at this time is also probably involved. L. digitata shows a similar increase in growth rates but since the response is only transient it does not appear on the graph when only monthly figures are considered.

iv EXOGENOUS P AND INTERNAL P

L. saccharina (Fig. 5 xiv) shows an overall positive correlation between exogenous P and internal P in the meristem. The pattern for the distal frond tissue is similar but extended since the internal P content is higher during the winter than in the meristem. The year is divided into 2 groups, an upper group, November - April, in the region of high external $\text{PO}_4\text{-P}$ and high internal P, and a lower group from May - September of low internal and external P.

In L. digitata (Fig. 5 xv) the pattern is similar to that shown for L. saccharina, with an overall positive

Figs. 5 xiv and 5 xv. Relationship of internal P content and seawater P concentration in *L. saccharina* and *L. digitata* respectively during 1980.

Fig. 5 xiv.

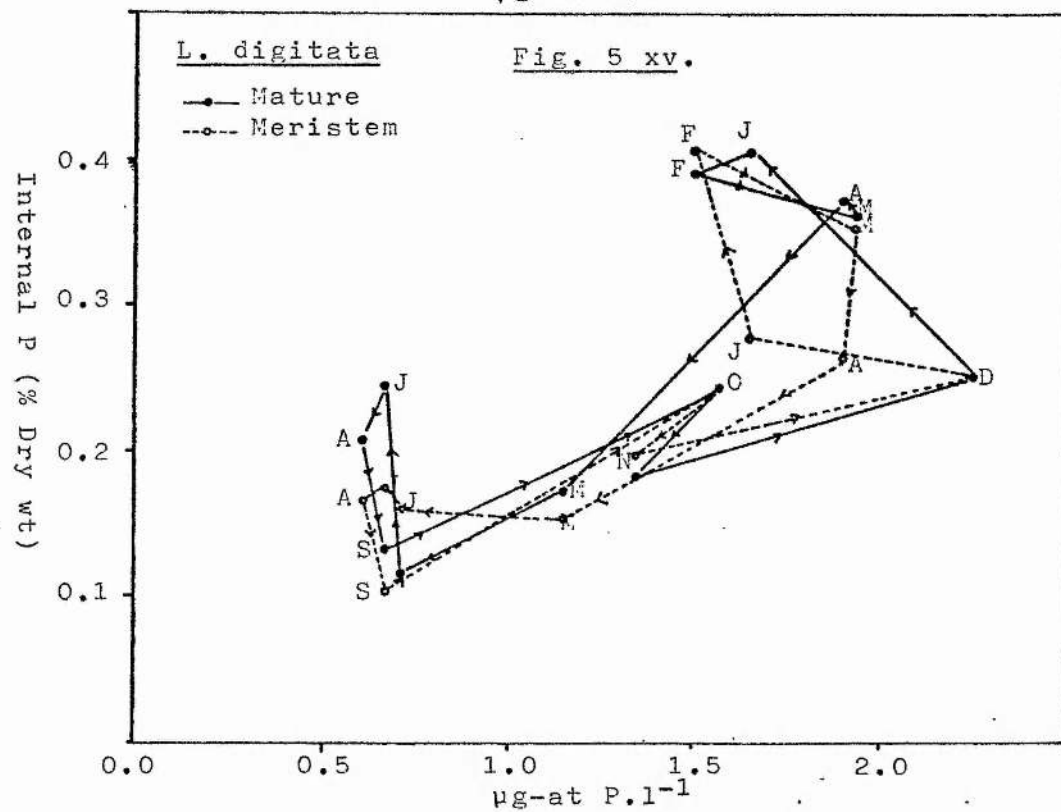
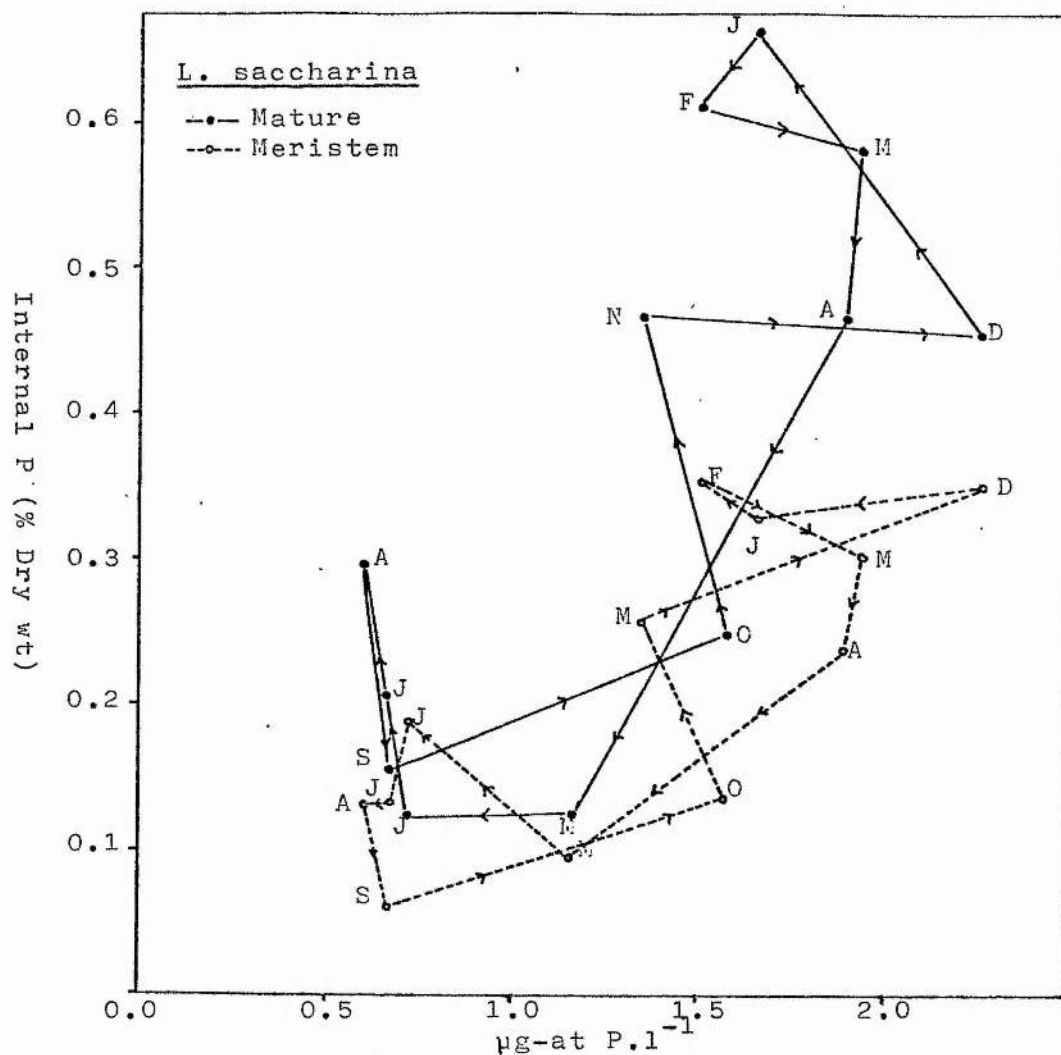


Fig. 5 xv.

growth rates at this time may result from an increase in available internal P, as external $\text{PO}_4\text{-P}$ also increases.

In L. digitata (Fig. 5 xvii) growth shows an overall negative correlation with internal P content. As with L. saccharina, the year may be divided into separate periods. From January-April the distal tissue P content exhibits a negative correlation with growth (February-May; $P < 0.10$, in the meristem), growth may be increasing during this period as available P is utilised. From May-September the distal tissue shows a positive correlation while the meristem is also positive, and from September - December both tissue areas show a negative relationship.

Internal P and growth are negatively correlated for L. saccharina and positively correlated for L. digitata in the mature tissue during the summer. Since it is the meristem which determines growth, the positive relationship shown by both L. digitata and L. saccharina probably provides a better measure of the interaction of internal P and growth, ie. growth decreases as internal P decreases. The distal tissue, although not actively growing appears to be accumulating P from the medium despite the low external phosphate concentrations (seen by the -ve correlation of L. saccharina): This effect may be an important consideration if $\text{PO}_4\text{-P}$ taken up by the distal tissue can be translocated to the actively growing meristem when internal P appears to be in short supply.

correlation and division of the year, in both the meristem and distal frond tissue.

Such a division of the year may indicate a difference in algal strategy during different parts of the year. The upper group, that is high seawater phosphate and high internal P content possibly represents the storage capacity of the alga, $\text{PO}_4\text{-P}$ is taken up in excess to requirements ie luxury consumption. The lower group, during the summer months may indicate that when $\text{PO}_4\text{-P}$ is in short supply, phosphate taken up from the medium is utilised immediately, there is no excess to requirements and therefore, little or no accumulation.

v . INTERNAL P AND GROWTH

Considering the 2 parameters in relation to L. saccharina (Fig. 5 xvi) the year is divided into 3 parts. In the distal frond tissue these are from January - April, May - August and September - January. During the first 2 periods internal P and growth exhibit a negative linear correlation ($P < 0.05$ and $P < 0.02$ respectively). However, during September - January, the relationship changes to a positive correlation ($P < 0.05$), with growth and internal both increasing. In the meristem, the pattern is slightly different; December - May (- correlation), June - September (+) and September - November (+). During the summer growth rates then decrease as internal P decreases. The positive linear correlation, September to November may suggest that the short-lived increase in

Fig. 5 xvi. Relationship between internal P and growth rate of *L. saccharina* during 1980 at St. Andrews.

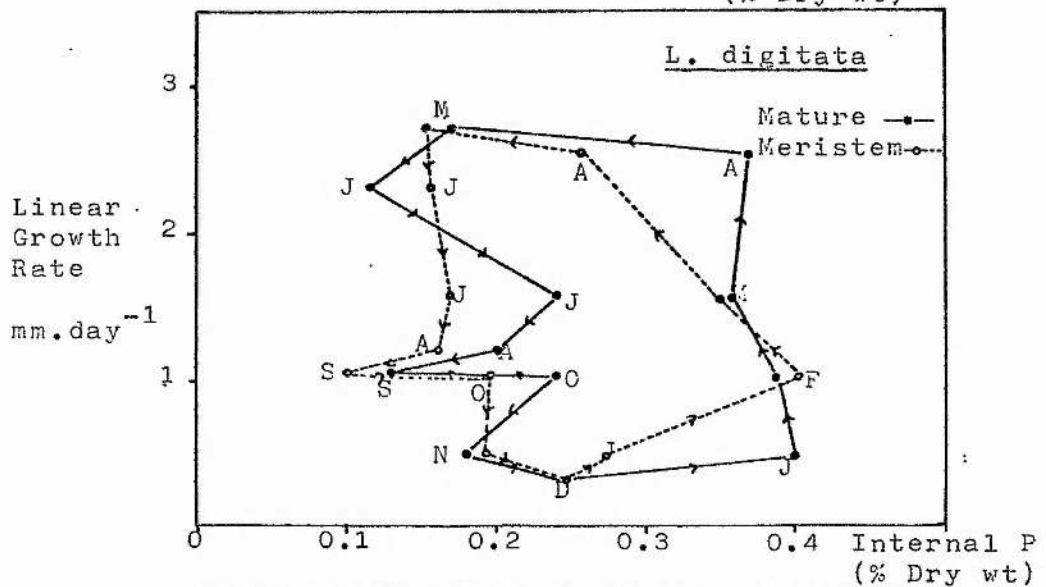
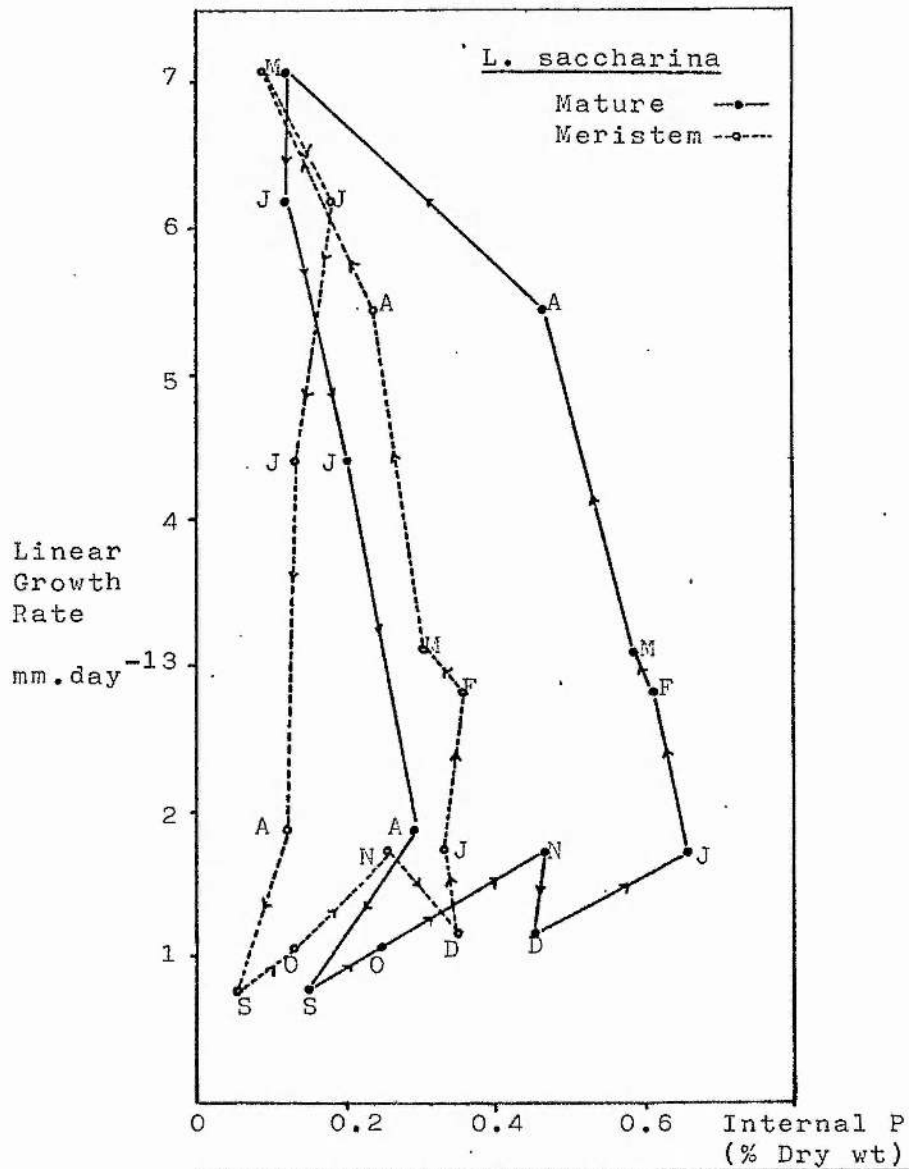


Fig. 5 xvii. Relationship between internal P and growth rate of *L. digitata* during 1980.

At St. Andrews Sewer and St. Andrews the ratio of the mean $\text{PO}_4\text{-P}$ concentration of the seawater during the winter months is $\frac{3.541}{1.884} = 1.880$.

In *L. digitata* the ratio of internal P at the 2 sites is $\frac{362.09}{261.58} = 1.384$. This figure is lower than is expected since external P and internal P are positively correlated and may suggest either external P is not being taken up efficiently at this time of year or that the internal P is being utilised more rapidly at Sewer than at St. Andrews. Data on the kinetics of P uptake (see later 5 vi a) indicates that exogenous P is taken up efficiently at this time and in conjunction with the apparent luxury consumption of P (during the winter months), this first possibility is unlikely to be correct. It is possible that rapid utilisation of internal P for growth is occurring, diluting tissue P and resulting in a lower than expected ratio of internal P.

The ratio of growth rates (maximum growth rates in May) of *L. digitata* at the 2 sites is $\frac{6.308}{2.741} = 2.30$.

If the growth rate was simply controlled by the level of $\text{PO}_4\text{-P}$ in the seawater and hence the P content of the tissue, this value would be similar to the ratio of internal p content and the ratio of seawater $\text{PO}_4\text{-P}$ concentration at the 2 sites. There is a discrepancy in the figures and a small rise in external P (ie Sewer is 1.88 x the P concentration at St. Andrews) results in a greatly increased growth rate. This increased growth rate cannot

be explained in terms of the internal P content or the seawater $\text{PO}_4\text{-P}$ concentration and some other factors eg nitrogen concentration, turbulence and the period of emersion may all be important and are discussed in more detail later.

In L. saccharina the situation appears to be slightly different. Here the ratio of external $\text{PO}_4\text{-P}$ concentration is 1.88; the ratio of internal P content is 2.13 and the ratio of growth rates at the 2 sites is 1.81.

The internal P content is higher than is predicted from the seawater phosphate concentration but the growth rates are less than expected from measurements of tissue P content. P is apparently being taken up in excess to immediate metabolic requirements ie there is luxury consumption of P. As with L. digitata, some other factors must be involved in determining frond growth rates of L. saccharina since growth is not directly related to seawater $\text{PO}_4\text{-P}$ concentration and tissue P content.

vi UPTAKE OF PHOSPHATE

a The effect of concentration on uptake of phosphate by discs of L. digitata in the light

(Uptake is measured as loss from the water over 48 hours in the light.)

The depletion curve (Fig. 5 xviii) is characteristic of Michaelis-Menten uptake, uptake follows saturation kinetics from 0-10 $\mu\text{g-at P/l}$ external concentration. The uptake parameters calculated from the curve are

Fig. 5 xviii. The effect of concentration on uptake of phosphate by discs of *L. digitata* over 48 hours in the light at 10 °C.

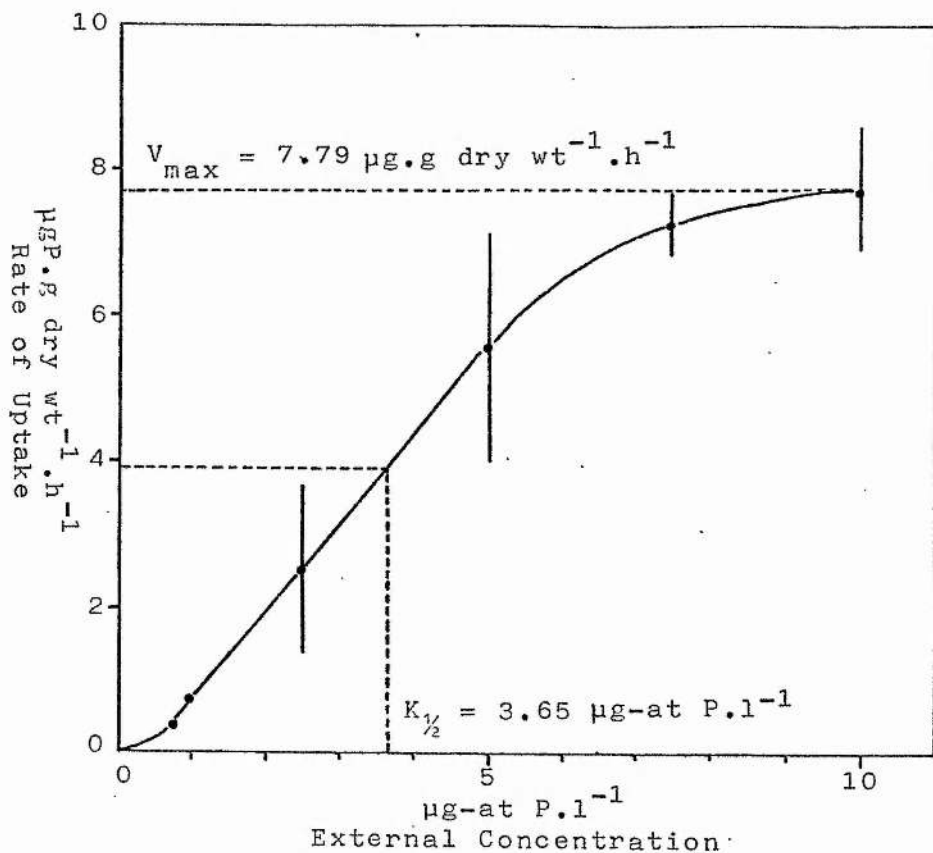
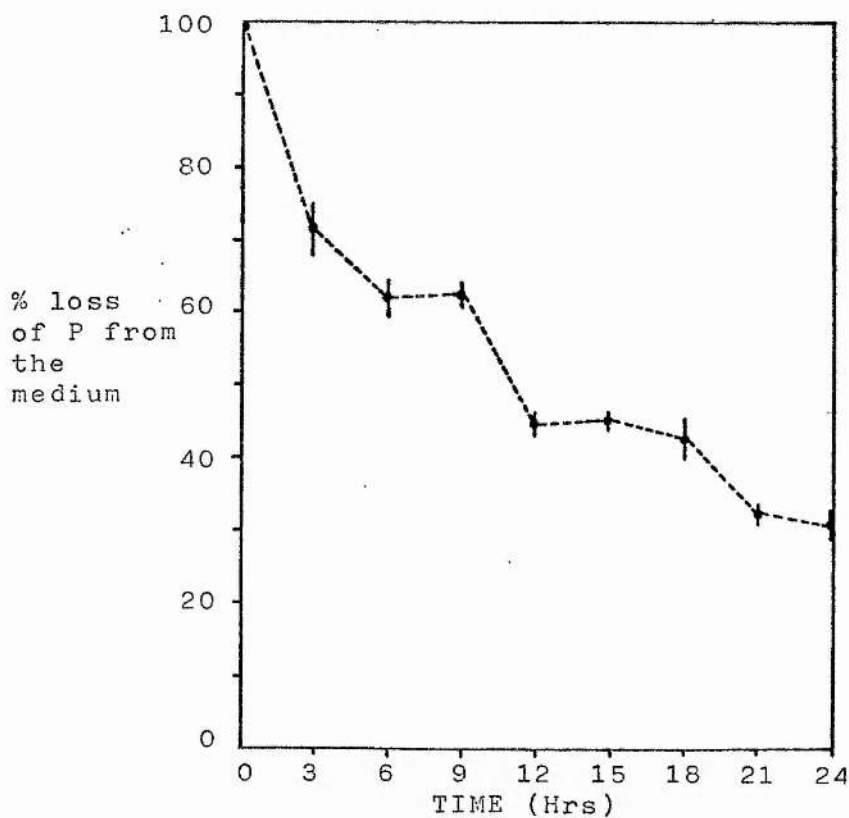


Fig. 5 xix. Time course uptake of phosphate by discs of *L. digitata* in the light over 24 hours. (Uptake measured as loss from the medium)



$V_{\max} = 7.79 \mu\text{g P.g dry wt}^{-1}.\text{h}^{-1}$. and site affinity or $K_{1/2} = 3.65 \mu\text{g-at P.l}^{-1}$.

Uptake of phosphate is saturated at phosphate concentrations which are higher than is normally found in situ during the winter. This excess of uptake capacity however, enables Laminaria to take advantage of any concentrated localised pockets of phosphate which are brought close to the frond surface. Winter seawater P concentrations occur in the linear part of the curve and presumably $\text{PO}_4\text{-P}$ is taken up every time it presents itself at the frond surface. But minimum summer P concentrations occur at the lowest point of the uptake curve, uptake is then at its least efficient at this time of year, diluteness of the substrate limiting supply to the frond surface.

b Time course uptake of phosphate by discs of
L. digitata in the light

Uptake of $\text{PO}_4\text{-P}$ is measured as loss from the medium over 24 hours in the light. (Fig. 5 xix).

After an initial loss of $\text{PO}_4\text{-P}$ from the water during the first 3 hours, uptake then proceeds at a steady rate. The overall rate of loss from the water over 24 hours is $2.74 \pm 0.11 \mu\text{g P.g dry wt}^{-1}.\text{h}^{-1}$. at an initial phosphate concentration of $1.26 \mu\text{g-at P.l}^{-1}$. Excluding the rapid loss during 0-3 hours, the overall rate of loss is $1.87 \mu\text{g P.g dry wt}^{-1}.\text{h}^{-1}$. The actual loss of phosphate from the medium is $56.38 \mu\text{g P.}$

The mean overall rate of increase of P by the tissue over 24 hours is $2.88 \pm 0.06 \mu\text{g P.g dry wt}^{-1}.\text{h}^{-1}$; an actual increase of $59.26 \mu\text{g P}$. This figure for gain by the tissue is not significantly different from the loss of $\text{PO}_4\text{-P}$ from the medium, suggesting that the extraction method for tissue P recovers almost 100% of that present and that there is little loss of $\text{PO}_4\text{-P}$ from the medium by absorption onto the glass of the flasks. No attempt was made, however, to estimate the proportion of the $\text{PO}_4\text{-P}$ gained by the tissue which is merely absorbed onto the mucilage produced by the discs and the proportion which is actually taken up by the cells.

vii GROWTH OF L. SACCHARINA IN PHOSPHATE ENRICHED SEAWATER IN SEPTEMBER

Small sporophytes of L. saccharina were grown for 20 days at ambient photoperiod (14 hrs Light: 10 hrs Dark) and seawater temperature (10°C) in ambient seawater ($0.3 \mu\text{g-at P.l}^{-1}$. and $0.5 \mu\text{g-at N.l}^{-1}$.) and P-enriched seawater ($3.0 \mu\text{g-at P.l}^{-1}$: $0.5 \mu\text{g-at N.l}^{-1}$) in September.

Results. P-enrichment in September had very little effect on linear growth rate, frond surface area increase or fresh weight increase over the course of the experiment. (Table 5 ii).

There may, however, be an effect of nitrogen limitation also operating since $\text{NO}_3\text{-N}$ is still at the minimum summer concentration. If these results are compared with those from the nitrogen-enrichment experiment during the

same period (Chapter 4, 4 v) the sporophytes supplied with high concentration of N in addition to high P showed only a limited response to the increased nutrient levels over this short-term experiment, but the results suggested that over the longer-term nitrogen-limitation may be important.

Table 5 ii. Growth of L. saccharina for 20 days at ambient photoperiod (14 hrs L), ambient seawater temperature (10°C) in Low-P ($0.3 \mu\text{g-at P.l}^{-1}$) and high-P ($3.0 \mu\text{g-at P.l}^{-1}$) seawater with $0.5 \mu\text{g-at N.l}^{-1}$. Mean \pm SE with 3 replicates/treatment.

Treatment	Mean Linear Growth rate (mm.day $^{-1}$)	% inc. in Surface Area	% inc. in Fresh wt.
Low P	2.28 ± 0.27	16.37 ± 4.24	27.77 ± 7.12
High P	2.37 ± 0.53	15.33 ± 4.92	22.89 ± 6.67
Significance	NS	NS	NS

From information of the internal P content of these sporophytes, it appears that in the Low-P treatment, growth is continued by utilisation of internal P, or at least, that present is diluted by growth and uptake is not sufficiently rapid to show as a net increase in internal P. Those in P-enriched seawater maintain growth at similar rates to those in the Low-P treatment, but they

accumulate P in the tissue (Table 5 iii).

Table 5 iii. Internal P content ($\mu\text{g P.100 mg dry wt}^{-1}$) at T=0 days and T=20 days in the meristem (0-5 cm) and the mature tissue (15-20 cm) of L. saccharina grown for 20 days in September. Significance indicated between Low-P and High-P. (See legend Table 5 ii)

Treatment	Meristem $\mu\text{g P.100 mg dry wt}^{-1}$	Mature Tissue $\mu\text{g P. 100 mg dry wt}^{-1}$
T=0	109.01 \pm 13.70	149.95 \pm 14.21
T=20		
Low-P	47.68 \pm 10.65	112.40 \pm 24.89
High-P	299.60 \pm 27.94	348.30 \pm 31.19
Significance	P<0.002	P<0.01

If P had a simple limitation effect then increasing external and internal P would result in an increased rate of growth which would be expected to be noticeable where there is such a significant ($P<0.01$) increase in internal P content over 20 days in both the meristem and mature frond tissue of L. saccharina in P-enriched seawater. Frond growth rates do not increase significantly and there are a number of possibilities which must be considered.

Some factor other than P is limiting growth. Nitrogen which has been discussed above is unlikely to be the sole

limiting factor, since there is little apparent effect on growth by adding high N and high P together in September (Chapter 4, 4 v). Trace elements are also unlikely to be limiting as these are added to the seawater during growth experiments. Light (photoperiod and irradiance) are still high in September and experiments were carried out at near saturating irradiances, and seawater temperature is also high and unlikely to be limiting growth at this time.

The experiment may be over too short a time period for changing growth rates to be evident. In situ seawater P begins to increase during early September (2nd or 3rd of September) but the transient increase in Laminaria growth starts after a lag period of about 3 weeks, between the 20-24th September. Since in situ growth rates are only measured fortnightly it may be assumed that growth rates actually increase during the fortnight preceding 20-24th September; the lag period is then considerably shorter than 3 weeks. Therefore, within 20 days some change in linear growth rate could be expected in the experimental plants, at least towards the end of the 20 day period. No such change is expressed and the linear growth rate is relatively constant over the 20 days. If a lag period is necessary in situ for Laminaria to build up the depleted P reserves then once again an increase in growth of the experimental plants would be expected since internal P increases to higher levels than

that measured in sporophytes in situ in late September/early October. Too short a time period for the experiment does not provide an adequate explanation for limited response of Laminaria to increased external P concentration.

The frond tissue may have undergone senescence and lost, to a large extent, its ability for cell enlargement and cell division whilst maintaining its uptake ability (evidenced by the significant increase in internal P). If senescence has occurred then the autumnal in situ growth increase may be transient because the frond has only a limited capacity for growth at this time (this is discussed in more detail later).

The results do indicate, however, that external and subsequently internal P are not solely responsible for the autumn growth increase shown by both L. saccharina and L. digitata.

DISCUSSION

Phosphate at the 4 sampling sites exhibits the typical seasonal fluctuation found in north Temperate waters. Maximum concentrations were recorded during the winter months, declining in the Spring (April) to minimum levels from May to September before increasing during the autumn to high winter levels. The decline in PO_4-P in the seawater coincides with the spring outburst of phytoplankton at St. Andrews (Richardson, 1969). Phosphate becomes locked up in the plankton (phyto- and zoo-), in

the macroalgae (as seen in this study) and in the sediments, Jitts (1959) showed that up to 90% of the phosphate in solution may be absorbed by estuarine silts. The low concentrations of nutrients in offshore surface waters during the summer is usually accompanied by some thermal stratification of the water, but there is little evidence for thermocline formation in shallow inshore water (Bowden, 1955; Lee, 1960) and at the 4 sampling sites there was probably insufficient prolonged periods of calm weather during the summer months for thermocline formation. The increase in $\text{PO}_4\text{-P}$ concentration during the autumn is thus, unlikely to be due to the lowering or breakdown of the thermocline, but increased turbulence would bring deeper P-rich water to the surface (eg in the English Channel the water below 20 m has high phosphate levels all year (Armstrong, 1954)) and increased run-off from the land would have an important local effect on phosphate levels. Domestic sewage (water from the Sewer outlet) has been shown to have a very high phosphate concentration. The effluent has a wide effect on nutrient levels at St. Andrews generally for although the effluent is rapidly dispersed and diluted from the outlet, the overall $\text{PO}_4\text{-P}$ concentration of the seawater at St. Andrews is raised above that at the 2 relatively unpolluted sites, Kingsbarns and Fifeness. The winter mean phosphate concentration at Kingsbarns and Fifeness is similar to that reported in other nutrient surveys around Great Britain, eg The

Menai Straits (Ewins & Spencer, 1967), Port Erin (Slinn, 1966), Liverpool Bay (Foster, Hunt, Pugh, Foster & Savidge, 1978), Cardigan Bay (Sykes & Boney, 1970), the English Channel (Atkins, 1923; Cooper, 1938; Armstrong; Butler & Boalch, 1972), West Coast of Scotland (Black & Dewar, 1949). During the summer phosphate at the 4 sites is never depleted to undetectable levels as is reported elsewhere (eg Cardigan Bay, Sykes & Boney, 1970) possibly as a result of sewage effluent at St. Andrews and Sewer and agricultural run-off at the other 2 sites; in addition water turbulence would continuously replenish the surface waters.

Laminaria accumulates P in the tissue during the autumn to a maximum in late winter (February/March). After this the P content declines rapidly to low levels in May/June, levels recover slightly during the summer but drop to a secondary minimum in September. The internal P content of the frond appears to follow changes in external P in both L. saccharina and L. digitata.

Laminaria appears to show luxury consumption of P during the winter (November - April) when phosphate is taken up in excess to immediate metabolic requirements, indicated by an increasing (actual and relative) tissue P content as external phosphate is at high concentrations. During this period, the P content of the frond may then indicate the storage capacity of the alga. The maximum P content of the tissue is similar at all 4 sites possibly

suggesting an upper limit to the amount of P which may be stored. If this is the case then plants in P-enriched areas would be expected to reach this maximum before those from low-P areas, although water turbulence, which is greater at Fifeness (because of the exposed nature of the site) would facilitate mineral nutrient uptake, and this site would not necessarily be later than St. Andrews or Sewer in reaching this value. However, there is no obvious pattern in the time at which the maximum is reached at the 4 sites and Sewer tends to be later than elsewhere.

Alternatively, this maximum figure may represent a threshold content, after which growth begins to increase rapidly and utilise the stored reserves. If this is the case then the tissue P content would be expected to be similar at the 4 sites at the point when spring growth is initiated in January. The results show a wide variation in P content between sites both in December (prior to spring growth initiation) and also in January indicating that levels of P alone are not responsible for the initiation of growth since if they were, St. Andrews and Sewer sporophytes would have begun their spring growth a month or 2 months previous to those at Kingsbarns or Fifeness. Such a threshold content may be important, however, in conjunction with other factors eg internal N, light and seawater temperature.

The similarity in maximum content may be explained in terms of the uptake capacity of the alga. If uptake systems in Laminaria are saturated at winter external

phosphate concentrations at the low-P sites (Kingsbarns and Fifeness) there is no nutritional advantage (with regard to P) for plants growing in $\text{PO}_4\text{-P}$ enriched areas, therefore, the amount of P taken up is similar at all 4 sites, resulting in similar maximum tissue content, assuming similar amounts and rates of P utilisation by plants at the 4 sites. However, with reference to the uptake kinetics of Laminaria, uptake is saturated between $7.5 - 10.0 \mu\text{g-at P.l}^{-1}$, a value far in excess to that found ecologically (except occasionally at St. Andrews Sewer). This theory therefore, does not provide an adequate explanation for the similar maximum tissue P content at the 4 sites.

Since exogenous and internal P are positively correlated, then Laminaria growing in P-enriched areas would be expected to have a higher internal P content. As indicated above, this is not true in the winter, where maximum levels are similar at the 4 sites. During the summer months when $\text{PO}_4\text{-P}$ is in short supply and phosphate taken up from the medium is utilised immediately and there is no excess to requirements, Laminaria from the enriched sites appear to be less limited by the external phosphate concentration than at Kingsbarns and Fifeness. Yet growth still remains low at these enriched sites suggesting limiting factors other than P are also important during the summer.

Both L. saccharina and L. digitata exhibit an inverse

relationship between growth and internal P from January - May. Since maximum growth occurs at minimum external P concentration, this suggests that internal P is far from limiting to growth down to 0.16% dry weight in L. digitata and 0.10% dry wt in L. saccharina (May values for St. Andrews meristem). This anomaly of maximum growth at minimum P probably indicates a time shift between uptake of P and utilisation for growth; hence internal P declines as it is utilised for growth, supporting growth for up to a month after the drop in both external and internal P. P declines before seawater P is depleted suggesting that P becomes limiting before maximum growth is reached.

P declines in the tissue as seawater phosphate drops in the Spring. There are two possible explanations for this, either

- a) It is lost by leakage
- or b) external P concentration limits uptake during the summer and reserves used for growth are not replenished.

The cell walls of Laminaria are rich in polymeric anions eg. alginates and sulphated alginic acids. As a result mobile PO_4^- is Donnan excluded (the electrical charge will repel anions). If the internal P is in the cell wall then it must be of a structural nature, but there is no evidence of this. Therefore, by inference, the phosphate is protoplasmic. P in the cell is an important component of nuclear proteins (DNA, RNA), ATP and cell membranes.

- a) The tissue concentration is the balance between influx (determined by the external concentration) and efflux (determined by the internal concentration) given a constant membrane potential. Efflux of PO_4^- from "ecological" concentrations is reported to be low relative to influx in Hydrodictyon africanum (Raven, 1975) and in Enteromorpha intestinalis (Black & Weeks, pers. comm.). The evidence presented suggests that loss to the water is low but while leakage needs to be tested it would be difficult experimentally, sterilization of the extracellular material to prevent microbial absorption of all effluxing PO_4^- could affect the Laminaria membranes adversely. P, once in the tissue is probably rapidly incorporated into organic compounds and polyphosphate and is unlikely to be leaked out in this form. Leakage, therefore, seems an unlikely explanation for the rapid decline in internal P.
- b) In April, $\text{PO}_4\text{-P}$ in the seawater drops rapidly to minimum levels during the summer months and it is suggested that internal P declines at this time as it is utilised to sustain the rapid spring growth rates. Summer phosphate concentrations occur at the very lowest part of the uptake curve (Rate v substrate concentration), uptake is linear at these external concentration indicating that the uptake system is far from saturated and is operating at its least efficient. $\text{PO}_4\text{-P}$ is presumably taken up whenever it appears, by convection or diffusion onto a P absorbing site on a periphery cell, $\text{PO}_4\text{-P}$ is presumably limited by the possibility of an ion arriving at the cell membrane. During the summer

therefore, supply limits uptake of phosphate. After the decline in seawater P growth rates are still high and P already in the tissue is diluted by growth since further efficient uptake of $\text{PO}_4\text{-P}$ is restricted by the low external concentrations. However, the sum total of P in the frond as a whole probably remains constant or may increase as uptake proceeds at a low rate, assuming negligible efflux of $\text{PO}_4\text{-P}$. It is difficult to measure the total P in the frond for a number of reasons:

- i) The increase in frond area with growth must be measured
- ii) as a result of the heterogeneity of the frond in terms of thickness it is difficult to select "representative" areas to allow extrapolation back to the frond as a whole
- iii) Continual erosion of the distal frond tissue results in loss of P.

In the more distal frond tissue internal P also declines very rapidly in the spring. In this region the cells are no longer actively dividing or enlarging and the P content is, therefore, not being diluted by growth in the same way as in the meristem. Assuming that there is negligible efflux then P must be declining as a result of basipetal translocation and ^{32}P has been shown to be transported in Laminaria from the distal frond tissue to the young tissue and the holdfast (Floc'h & Penot, 1971; 1972; 1974; 1976; 1978). The distal tissue may then represent an important reserve of P during the spring and summer.

Most of the evidence presented indicates that levels of phosphate in the seawater during the summer are indirectly limiting to growth of Laminaria. Luxury consumption of P occurs during the winter and the internal P built up is utilised to sustain growth at high rates for up to 1½ months after the depletion of seawater $\text{PO}_4\text{-P}$, evidenced by the rapid depletion of internal P to minimum levels in May. The uptake system is limited by the supply of phosphate to the cell membrane and internal P levels, diluted by growth are not replenished sufficiently rapidly by uptake. P, therefore, becomes limiting to cell division and as a result growth rates decline.

But is P the only constraint on growth during the summer or are some other factors also involved? Internal P is seen to increase slightly during the summer. Such an accumulation would only occur if some other factor(s) then becomes limiting to growth; growth is slow and there is little requirement for P and uptake is then in excess to immediate requirements. If P was the sole constraint, accumulation would not occur but growth rates might be seen to increase in response to this increased availability of P. Similarly if P is the only limitation to growth then addition of phosphate to the medium of depleted plants would be expected to give increased growth rates. However, this was found not to be the case (see Interaction N + P), where growth rates of L. saccharina were not enhanced significantly despite a rapid and significant accumulation of internal P in P-enriched seawater.

If we assume that some other factor is limiting growth during the summer, it is unlikely to be either light (photoperiod or irradiance) or seawater temperature, but as indicated in the previous chapter the concentration of nitrogen in the seawater appears to be limiting during the summer months and this may have a significant effect in conjunction with phosphate concentration. The possibility of tissue senescence must also be considered since L. digitata and L. saccharina show only a transient growth increase as exogenous P (and N) increase in September whilst light and temperature are still high. The interaction of N and P and tissue senescence are discussed further in the next chapter.

To summarise: it is therefore, suggested that phosphate in conjunction with other factors (possibly N) limits the growth of Laminaria in the late spring and summer. Once growth has slowed, after May, P is no longer the major limitation and internal P increases slightly as a result. The low external P concentration during the summer means that uptake of phosphate is limited by supply of the ions to the cell surface. The low external and internal P limits growth indirectly and that, in conjunction with other factors may bring about senescence of the frond tissue. During the winter Laminaria shows luxury consumption of P and these accumulated reserves are utilised to support growth for up to 1½ months after the spring decline in seawater P.

CHAPTER 6

INTERACTION OF NITROGEN AND PHOSPHATE

INTRODUCTION

Evidence presented as to internal reserves and the uptake kinetics of nitrate and phosphate in the previous 2 chapters suggest that N and P are at concentrations which are limiting summer growth of L. digitata and L. saccharina. The summer decline in linear growth rates follows the depletion of seawater N and P after a lag period of about one month during which time internal reserves are utilised, and although this close association suggests a direct causal interaction there is relatively little evidence in the literature to substantiate this. At St. Margarets Bay, Nova Scotia Chapman & Craigie (1977) were able to prevent the spring decline in growth rates in L. longicruris by enrichment with $\text{NO}_3\text{-N}$ in situ (seawater $\text{PO}_4\text{-P}$ did not decline markedly during the summer at this location) and enrichment with both nitrate and phosphate in the laboratory resulted in increasing growth rates (rather than the decline as in the controls) during the summer in Alaria esculenta (Buggeln, 1974). Buggeln (1974) showed that N and P were limiting growth during the summer but he did not correlate seasonally varying growth rates with the precise timing of nutrient fluctuations in the sea. However, despite extensive research on the British Laminariales, enrichment with nitrate and phosphate during the spring and summer has

not previously been attempted.

If seawater nutrient decreases are directly responsible for the spring decline in Laminaria growth, enrichment during the summer would be expected to result in increased Laminaria growth. However, data presented in the previous 2 chapters suggests that enrichment in late summer (September) did not produce the increase in growth rates predicted from the external nutrient concentration supplied and the internal tissue N and P content. It was tentatively suggested that this failure to realise their spring growth potential may indicate that the tissue has lost the spring-time ability for rapid and extensive cell division, that is, the tissue has become senescent (either endogenously or exogenously produced). Since temperature and light are unlikely to be limiting Laminaria growth at this time (September) senescence may be a real possibility.

Enrichment experiments were therefore carried out at 2 different times, in mid-summer (June/July) and in late summer (September) to investigate whether senescence does occur and if so, whether it is a rapid effect ie within the first 2 months of seawater nutrient depletion or whether it occurs more slowly (ie up to 5 months after nutrient depletion). Senescence in this instance is recorded as a loss of cell division capacity whilst retaining the ability for nutrient uptake.

The immediate effects of the spring nutrient decline

were investigated by enrichment experiments in April as seawater N and P declined in order to ascertain:

- i Whether the decline in seawater N and P is causal to the spring decline in Laminaria growth rates, and
- ii if senescence effects are possibly apparent later in the summer (September) there may also be immediate effects as seawater nutrients decline in April.

The immediate senescence effects (ii above) were investigated by measurements of photosynthetic capacity (that is, the maximum photosynthetic rates possible by the alga given 'ideal' conditions of temperature, light and bicarbonate) during the spring enrichment experiments.

The results are, therefore, divided into 3 sections,

- i Growth and the Spring decline in nutrients
- ii Growth during mid-summer
- iii Growth during late summer - September

RESULTS

i GROWTH AND THE SPRING DECLINE IN NUTRIENTS

- a Experiment to investigate whether the decline in N and P in the seawater in April causes the decline in growth rate of *L. digitata*

17 small *L. digitata* sporophytes were used with the fronds cut to 15 cm in length and holes (0.25 cm diameter) punched at 5 and 10 cm from the transition zone. The

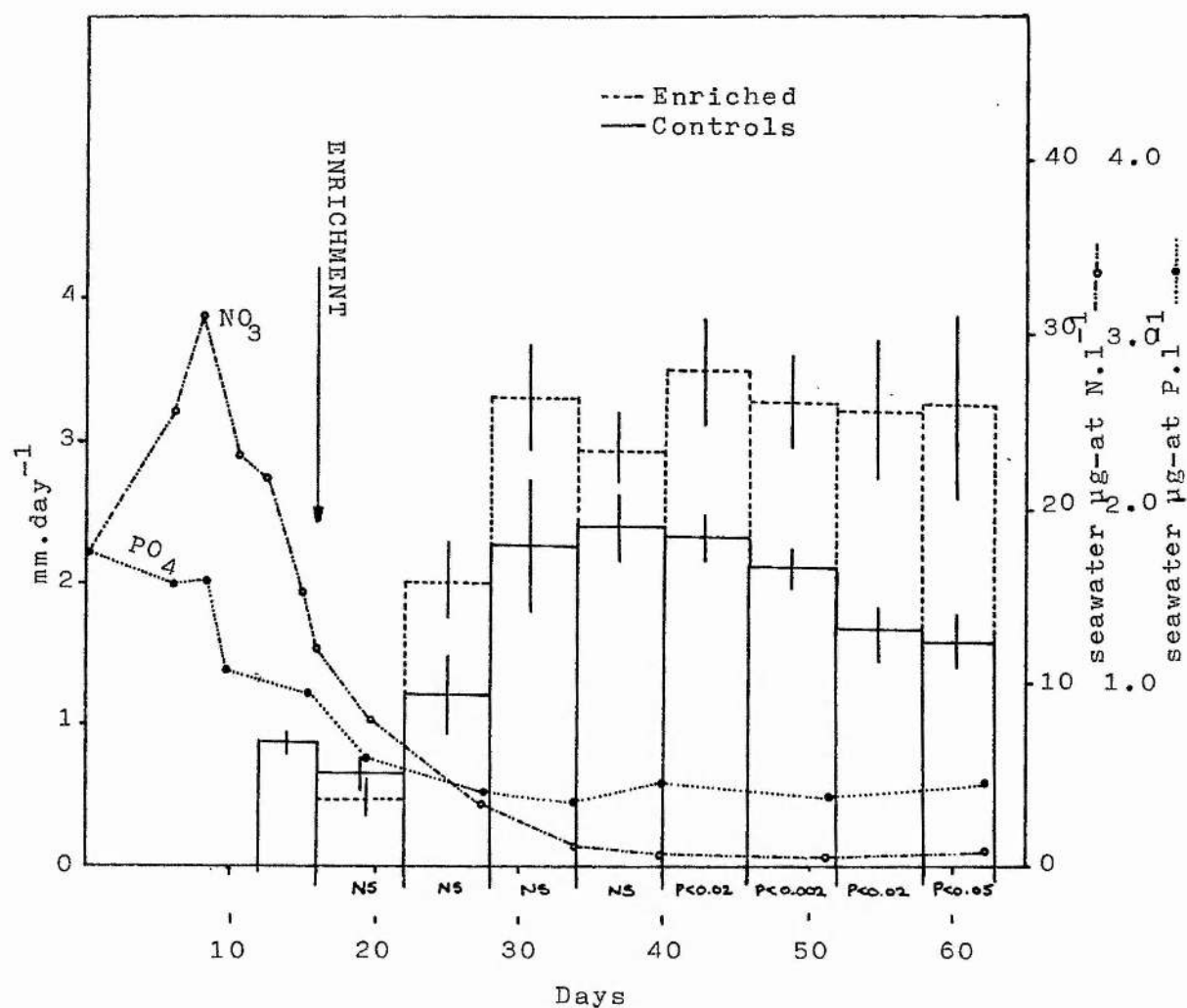


Fig 6 i. Growth of *L. digitata* in ambient seawater (Control) and seawater enriched with NO_3 and PO_4 ($15 \mu\text{g-at N/l}$, $3.0 \mu\text{g-at P/l}$) in the spring at ambient seawater temperature and ambient photoperiod. Enrichment began as seawater nitrate and phosphate declined.

seawater was analysed for $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$; when these declined in April, 3 sporophytes were removed for photosynthetic measurements (see 6 i c) the remaining 14 were divided into 2 groups and grown with aeration at the initial ambient seawater temperature (5°C) and ambient photoperiod (12.25 hours Light); the temperature and photoperiod were then adjusted weekly to maintain ambient levels, in

- a ambient seawater (low N and P controls)
- b Seawater enriched with nitrate and phosphate ($15.0 \mu\text{g-at N.l}^{-1}$; $3.0 \mu\text{g-at P.l}^{-1}$)

The seawater N and P concentration is shown in Fig. 6 i; on day 0 (25/3/81) nitrate and phosphate are at high winter concentrations, but they drop sharply after 8 days to minimum levels by the end of April and throughout May. Enrichment begins on day 16 and further nutrients are added every 2 days.

Linear growth rates (Fig. 6 i). In the controls, L. digitata growth rates increase to a peak between day 34 - 40 (approximately one month after the depletion of N and P in the seawater) and this spring maximum frond elongation rate is similar to that expressed by L. digitata in situ ($2.41 \pm 0.27 \text{ mm.day}^{-1}$ in the laboratory and $2.79 \pm 0.45 \text{ mm.day}^{-1}$ in situ at St. Andrews). The enriched L. digitata show a greater increase in linear growth rates, which level off at 3.26 mm.day^{-1} ; the elongation rates do not decline significantly after the

peak at 40-46 days. By the end of the experiment (T=63 days) the L. digitata in N+P enriched seawater were growing at over twice the rate of those in ambient seawater at 3.26 mm.day^{-1} and 1.57 mm.day^{-1} respectively.

The enriched sporophytes appeared healthy and darkly pigmented, but the controls were paler, the fronds were fragile and readily eroded at the distal end by water movement in the perspex tubes. Chapman & Craigie (1977) noted a similar effect in L. longicruris enriched in situ with $\text{NO}_3\text{-N}$. Measurements of the pigment content (chlorophyll and fucoxanthin) in L. digitata were not made but it appears that external N and P limitation may reduce pigment synthesis and the pigment present is then diluted by growth. Quantitative measurements are necessary to establish whether this is the case but Chapman, Markham & Luning (1978) recorded an increase in chlorophyll content with increasing nitrate concentration up to approximately $4.52 \mu\text{g-at N.l}^{-1}$ in L. saccharina.

i GROWTH AND THE SPRING DECLINE IN NUTRIENTS

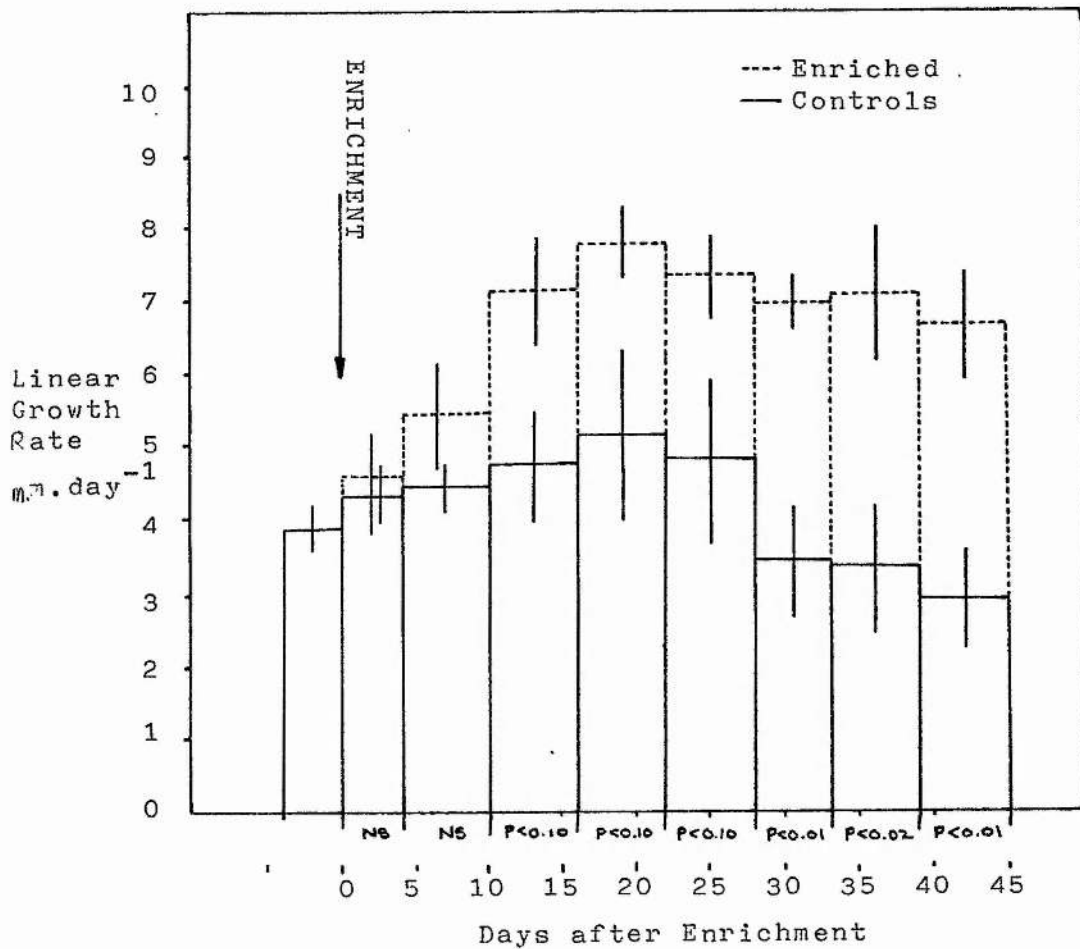
- b Experiment to investigate whether the spring decline in N and P in the seawater causes the decline in growth rate of L. saccharina

The decline in seawater N and P is shown in Fig. 6 i; the experimental details are similar to those for L. digitata described above and enrichment begins on the same date.

Linear growth rates. In ambient (low N+P) seawater

Fig 6 ii. Growth of *L. saccharina* (mm.day⁻¹) in ambient seawater (Control) and seawater enriched with NO₃ and PO₄ (15 µg-at N/l, 3.0 µg-at P/l).

(Mean \pm SE with 5 replicates/treatment)



linear growth rates of the control plants increase to a peak 16-22 days after the decline in seawater N and P (Fig. 6 ii). The enriched sporophytes increase steeply to a maximum at the same time (ie 16-22 days after enrichment) but the rapid growth rates are maintained during the course of the experiment and by the last measurement (39-45 days after enrichment) they show a linear growth rate of $6.70 \pm 0.74 \text{ mm.day}^{-1}$ compared to $2.90 \pm 0.68 \text{ mm.day}^{-1}$ in the controls.

It, therefore, appears that the spring growth decline is prevented in these experiments in both L. saccharina and L. digitata suggesting that in situ the spring decline in seawater N and P is causal to the observed spring decline in Laminaria growth rates which occurs one month following this nutrient decline.

i GROWTH AND THE SPRING DECLINE IN NUTRIENTS

c The effect of the spring nutrient decline on Photosynthetic Capacity of L. digitata

The photosynthetic capacity (or potential), that is, the net photosynthetic rate given 'ideal' conditions (light intensity above saturation, adequate bicarbonate, maximum temperature found in the sea - 15°C) of different frond areas was measured on day 16 (prior to enrichment) and at the end of the experiment (day 63) to investigate whether this changes on nutrient depletion.

Initially, photosynthetic measurements were made from three areas of the frond:

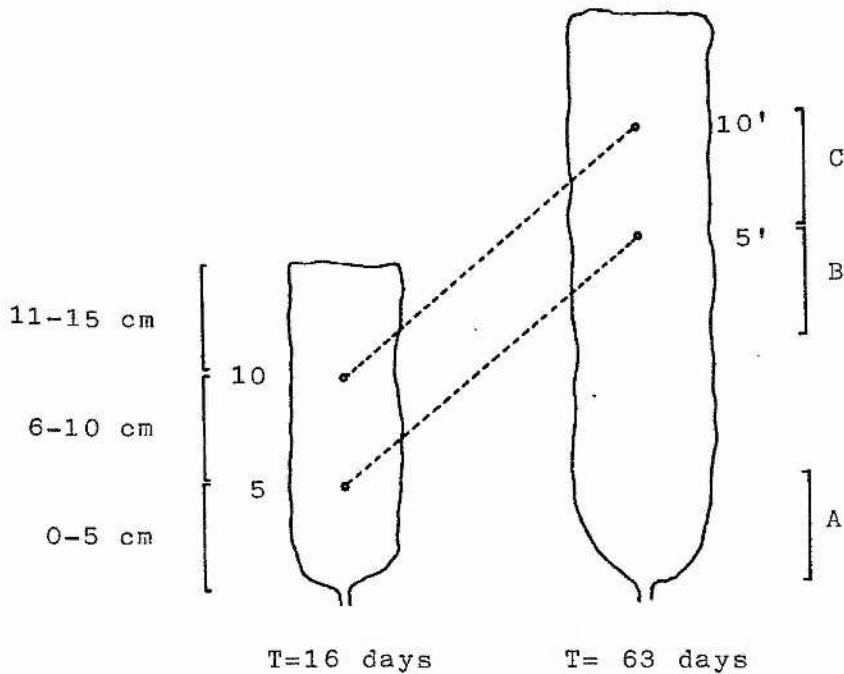


Fig 6 iii. *L. digitata* frond prior to enrichment (T=16 days) and at the end of the experiment (T=63 days). The areas used for photosynthetic measurements at T=16 are 0-5 cm, 6-10 cm and 11-15 cm. At T=63, holes originally punched at 5-10 cm remain 5 cm apart and tissue used for photosynthetic measurements at B is equivalent to 0-5 cm (T=16) and tissue at C = 6-10 cm. A = basal 5 cm (meristem).

- i at the meristem (basal 0-5 cm)
- ii frond 6-10 cm from the transition zone
- iii more distal tissue between 11-15 cm.

At the end of the experiment comparable areas were selected for measurement:

- i at A, meristem 0-5 cm
- ii the tissue originally at 0-5 cm moves along the frond with growth to become tissue at B (5 cm basal to the hole punched originally at 5 cm).
- iii the tissue at 6-10 cm in the same way becomes tissue at C. During growth in all plants holes originally punched at 5 and 10 cm from the transition zone remained 5 cm apart (thus, 6-10 cm is equivalent to C). (Fig. 6 iii)

The more distal tissue was not used for final photosynthetic measurements because of the high degree of erosion in the control plants.

Results. The photosynthetic capacity of the meristem (basal 5 cm) does not change significantly over the course of the experiment (Table 6 i) in either the controls or the enriched sporophytes. However, in the more distal frond tissue, at B and C, the net photosynthetic rates are significantly lower in the controls than in the enriched treatment (At B, $P < 0.05$ and at C, $P < 0.05$).

The photosynthetic capacity increases as the tissue moves distally away from the meristem, ie 6-10 cm has a

Table 6 i. Net photosynthetic rates ($\mu\text{l O}_2 \cdot \text{g dry wt}^{-1} \cdot \text{h}^{-1}$) of different frond areas of L. digitata prior to enrichment (T=16 days) and at the end of the experiment (T=63 days). Small sporophytes were grown in a) ambient seawater (control) and b) seawater enriched with $\text{NO}_3\text{-N}$ ($15 \mu\text{g-at N} \cdot \text{l}^{-1}$) and $\text{PO}_4\text{-P}$ ($3.0 \mu\text{g-at P} \cdot \text{l}^{-1}$). Measurements were made in saturating white light at 15°C over 3 hours. See text for explanation of frond areas. (3 replicates/treatment. Mean \pm SE)

Frond Area (cm)	T=16 days $\mu\text{l O}_2 \cdot \text{g dry wt}^{-1} \cdot \text{h}^{-1}$	
0-5	255.78 \pm 145.28	
6-10	1118.64 \pm 244.45	
11-15	1082.14 \pm 67.99	
Frond Area	T=63 days $\mu\text{l O}_2 \cdot \text{g dry wt}^{-1} \cdot \text{h}^{-1}$	
	CONTROL	ENRICHED
A	247.83 \pm 175.11	206.10 \pm 173.65
B	174.65 \pm 203.76	852.06 \pm 42.25
C	287.92 \pm 284.62	1420.29 \pm 155.96

higher net photosynthetic rate than 0-5 cm (at T=16) and in the enriched treatment as 0-5 cm (at T=16) becomes B (by T=63) there is a significant increase in photosynthetic rate ($P < 0.02$). However, in the controls such a dramatic increase does not occur and the photosynthetic capacity of B remains very similar to that of the meristem. Tissue which was originally at 6-10 cm and which has become tissue at C by T=63 increases in the enriched (not significantly) but decreases significantly ($P < 0.10$) in the control treatments.

It, therefore, appears that photosynthetic capacity of the frond tissue is significantly reduced after external N and P decline to low summer levels; this loss of photosynthetic capacity is prevented by maintaining high external N and P concentrations.

Several factors may explain the low net photosynthetic rates in the meristem as compared to the more distal tissue:

- i there may be less chlorophyll per unit dry weight (chlorophyll measurements were not made)
- ii the meristem is thicker than tissue further along the frond so there is relatively more non-photosynthetic to photosynthetic tissue
- iii there is a diversion of metabolism from

photosynthesis to growth systems

- iv active cell division occurring in this region may result in higher respiratory demand and hence net photosynthesis is reduced.

However, the respiration rates were not found to be higher in this region and i-iii may provide a better explanation.

To summarise; Growth and the Spring Decline in nutrients. The decline in seawater N and P causes a slowing of growth in L. saccharina and L. digitata after a lag period of about one month. Enrichment, to maintain mean winter concentrations of PC_4 -P and NO_3 -N prevents this decline.

The photosynthetic capacity of L. digitata meristem remains constant with time (over the course of the experiment) and regardless of the external N and P concentration. In the enriched L. digitata, the photosynthetic capacity increases with distance from the transition zone, but this is not observed in the control plants. In these plants, the tissue on leaving the meristem (0-5 cm) does not significantly increase its photosynthetic capacity but instead remains constant and low in region B. There is also a dramatic decline in the photosynthetic capacity of the older tissue (6-10 cm and C). It appears that depletion of seawater N and P induces a very rapid senescence effect (or loss of photosynthetic

capacity or potential) on the frond tissue of L. digitata, and senescence is prevented (or at least delayed) when nitrate and phosphate are maintained at high (winter) concentrations. This data provides no information as to the reversibility of senescence and a test of reversibility by addition of N and P would be desirable later in the year (mid-summer and autumn).

ii GROWTH DURING MID-SUMMER

a Experiment investigating whether nitrate and/or phosphate are limiting to growth of L. saccharina during June/July

L. saccharina (whole plants) were grown in perspex tubes for 20 days during June/July at ambient seawater temperature ($13^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$) and ambient daylength (17.5 hours Light) at 4 nutrient treatments:

a	Low N + Low P	$0.9 \mu\text{g-at N.l}^{-1}$: $0.5 \mu\text{g-at P.l}^{-1}$ (Control, ambient seawater)
b	Low N + High P	$0.9 \mu\text{g-at N.l}^{-1}$: $1.8 \mu\text{g-at P.l}^{-1}$
c	High N + Low P	$7.5 \mu\text{g-at N.l}^{-1}$: $0.5 \mu\text{g-at P.l}^{-1}$
d	High N + High P	$7.5 \mu\text{g-at N.l}^{-1}$: $1.8 \mu\text{g-at P.l}^{-1}$

Results. The linear growth rate of the frond is significantly increased above the control only when both N and P are supplied at high concentrations ($P < 0.05$), (Table 6 ii) and similarly, frond surface area increases significantly only when L. saccharina is enriched with high nitrate and high phosphate simultaneously (Table 6 ii),

(Low N, Low P; $P < 0.10$; Low N, High P; $P < 0.05$; High N, Low P; $P < 0.02$).

Table 6 ii. The mean linear growth rate ($\text{mm} \cdot \text{day}^{-1}$) anterior to the hole originally punched at 20 cm, and the % increase in surface area (in parentheses) of L. saccharina grown for 20 days at 13°C in June/July. (Mean \pm SE with 3 replicates/treatment)

		$\text{PG}_4\text{-P}$ ($\mu\text{g-at P} \cdot \text{l}^{-1}$)	
		0.5	1.8
$\text{NO}_3\text{-N}$ ($\mu\text{g-at N} \cdot \text{l}^{-1}$)	0.9	4.68 ± 1.09	4.65 ± 0.78
		(37.55 ± 16.30)	(49.18 ± 7.29)
	7.5	4.17 ± 0.33	6.68 ± 0.68
		(46.22 ± 4.83)	(82.11 ± 7.05)

Growth rates of plants with either N or P or both at low concentrations, are similar to those expressed by plants in situ ($4.40 \pm 0.87 \text{ mm} \cdot \text{day}^{-1}$ at St. Andrews) but enhanced growth is similar to maximum spring rates.

The dry weight of L. saccharina also increases significantly only when both N and P are at maximum concentrations ($P < 0.10$, Table 6 iii).

Internal reserves

Internal N. In the mature tissue (Table 6 iv) the pattern of internal N content is as expected, L. saccharina supplied with high N have a higher internal N content than

Table 6 iii. % increase in dry weight of L. saccharina grown for 20 days at photoperiod (17.5 hours Light) at 13 °C in June/July. (Mean \pm SE)

		$PO_4\text{-P}$ ($\mu\text{g-at P.l}^{-1}$)	
		0.5	1.8
$NO_3\text{-N}$ ($\mu\text{g-at N.l}^{-1}$)	0.9	52.33 \pm 16.58	59.53 \pm 8.77
	7.5	62.48 \pm 9.52	104.95 \pm 12.50

those at minimum N. In the treatments with high N the protein content increases at the expense of the organic (non-protein)-N and the inorganic fractions, ie there is a 70% increase in protein but a decline of 67% and 63% of non-protein-N and inorganic-N respectively, at high N + High P after 20 days. This decline may reflect synthesis of the 2 fractions into protein or translocation of the readily utilisable N fractions away from the mature tissue. At Low N + Low P there is a dramatic decline in internal (total) N, possibly as a result of basal translocation at a time when uptake of N from the medium cannot supply growth demands.

In the meristem the total N content increases only when both N and P are supplied at high concentrations and at low N + low P there was a dramatic decline in N content. The organic (non-protein)-N and inorganic N fractions decrease under all 4 treatments but the protein content

increases (possibly as a result of synthesis from non-protein and inorganic N) in all but the Low N + Low P treatments, with the greatest increase (141%) in High N + High P. The total N content of the meristem in High N + Low P would be expected to be higher than Low N + High P, but this is not the case (values are not significantly different). This is may be explained as a result of export from the distal tissue at Low N + High P to maintain high N levels in the meristem.

Table 6 iv. Total N content ($\mu\text{g N.100 mg dry wt}^{-1}$) of discs of tissue (2.5 cm diameter) cut from the meristem (2 cm) and mature tissue (20 cm) of L. saccharina grown for 20 days in June/July at 13 °C, photoperiod (17.5 h L).

	$\mu\text{g N.100 mg dry wt}^{-1}$	
	MERISTEM	MATURE TISSUE
T=0 Days	650.97 \pm 125.12	617.46 \pm 397.96
T=20		
($\mu\text{g-at.1}^{-1}$)		
N : P		
0.9 : 0.5	262.70 \pm 130.58	158.37 \pm 66.85
0.9 : 1.8	524.22 \pm 81.49	313.33 \pm 42.71
7.5 : 0.5	589.29 \pm 169.14	768.75 \pm 201.32
7.5 : 1.8	959.09 \pm 331.57	893.91 \pm 154.22

However, there are problems in interpretation of discs cut from the meristem and the mature tissue. Any losses or decline in internal N may merely reflect that photo-

synthesis has diluted the N content/unit dry wt more in that particular treatment than the others. This dilution would be a large scale effect detectable in the results as too would translocatory losses and any decline in internal N can be interpreted as either or both of these factors operating since N efflux is unlikely to explain large changes in relative N content.

Internal P.

Table 6 v. Internal P content ($\mu\text{g P.100 mg dry wt}^{-1}$) of the meristem and mature tissue of L. saccharina grown for 20 days in June/July at 13 °C, photoperiod (17.5 h L).

	$\mu\text{g P.100 mg dry wt}^{-1}$			
	MERISTEM		MATURE TISSUE	
T=0 Days	55.59	\pm 6.83	48.83	\pm 3.99
T=20 Days				
($\mu\text{g-at.l}^{-1}$)				
N : P				
0.9 : 0.5	26.44	\pm 2.40	23.70	\pm 4.44
0.9 : 1.8	219.24	\pm 19.63	224.59	\pm 7.43
7.5 : 0.5	34.53	\pm 6.15	21.40	\pm 4.07
7.5 : 1.8	242.02	\pm 19.54	235.01	\pm 15.90

When the external $\text{PO}_4\text{-P}$ concentration is low the tissue P content of the meristem and the mature tissue declines over the course of the experiment, (Table 6 v) and similarly when external P concentration is high tissue

P increases. In both the meristem and mature tissue the presence of high or low external N concentration does not have a significant effect on the P content of the tissue.

The fall in $\mu\text{g P} \cdot 100 \text{ mg dry wt}^{-1}$ is assumed to occur by dilution of P with P free dry matter rather than efflux of soluble PO_4 -compounds particularly in the meristem but also in the mature tissue where translocation basally may also be important. The results appear to indicate that summer external PO_4 -P concentrations are at levels limiting to growth.

Summary. Both nitrate and phosphate concentrations appear to be limiting growth of L. saccharina during the summer (June/July); enrichment with both N and P ($7.5 \mu\text{g-at N} \cdot \text{l}^{-1}$ and $1.8 \mu\text{g-at P} \cdot \text{l}^{-1}$) significantly increases the linear growth rate, dry weight and frond surface area. Individually, high concentrations of N or P do not enhance growth.

Interpretation of internal reserves is difficult, but relative internal P content follows changes in external P concentration and therefore, increases with high external P concentration and decreases with low external P levels. Similarly, relative internal N with external nitrate concentration. Low external N or P or both treatments are utilising tissue N and/or P to maintain the observed growth rates and extending the experiment would probably result in further decreases in growth rate, as is observed in situ.

However, the growth rates of these L. saccharina

enriched with high N+P in June/July is less than is predicted from the supply concentration as compared with the controls.

The ratio of the supply concentration (N+P) is $\frac{9.3}{1.4} = 6.64$. The tissue content, which is determined by the external supply concentration (see Chapters 4 and 5) in the mature tissue is similar to the ratio of supply concentration (6.20) whereas utilisation during growth means that this figure is lower in the meristem (4.15). However, the ratio of growth rates over 20 days of L. saccharina enriched with maximum external NO_3 and PO_4 to that of the controls is $\frac{6.65}{4.68} = 1.42$.

Therefore, both the ratio of supply concentration and tissue content are considerably higher than the ratio of the growth rates and, assuming adequate mixing in the growth tubes, the growth potential is not realised, indicating the possibility of tissue senescence after 2 months of nutrient depletion.

iii GROWTH DURING LATE SUMMER - SEPTEMBER

a Experiment investigating the growth of discs of L. digitata on a range of nitrate and phosphate concentrations in September

Discs of L. digitata were grown for 20 days at ambient photoperiod (14 hours Light) and at ambient seawater temperature (14.75°C) under 9 nutrient combinations; combinations of 0.5, 5.5 and $10.5 \mu\text{g-at N.l}^{-1}$ (as nitrate) and 0.5, 2.5 and $4.5 \mu\text{g-at P.l}^{-1}$. Ambient seawater (Control)

is $0.5 \mu\text{g-at N.l}^{-1}$ and $0.5 \mu\text{g-at P.l}^{-1}$.

Results

The % change in dry weight is not significantly different between all concentrations of NO_3 and PO_4 , although the greatest increase occurs at 10.5 N : 4.5 P. Intermediate combinations (5.5 N : 2.5 P) appear inhibitory as is maximum P with low NO_3 concentrations (Table 6 vi).

Table 6 vi. % change in dry weight (in parentheses) and mean disc area/treatment (mm^2) of discs of L. digitata grown for 20 days on a range of nitrate and phosphate concentrations in September at 14.75°C and with a photo-period of 14 hours Light.

		$\text{NO}_3\text{-N } (\mu\text{g-at N.l}^{-1})$		
		0.5	5.5	10.5
$\text{NO}_3\text{-N } (\mu\text{g-at N.l}^{-1})$	0.5	(7.32 \pm 2.02) 547.30 \pm 6.28	(6.15 \pm 2.80) 546.42 \pm 5.31	(8.96 \pm 2.23) 550.92 \pm 6.73
	2.5	(5.31 \pm 5.24) 541.90 \pm 5.72	(3.06 \pm 5.91) 546.92 \pm 6.50	(9.38 \pm 2.44) 548.41 \pm 7.03
$\text{PO}_4\text{-P } (\mu\text{g-at P.l}^{-1})$	4.5	(5.22 \pm 4.66) 530.75 \pm 4.61	(6.02 \pm 4.86) 550.83 \pm 8.00	(11.70 \pm 5.25) 560.52 \pm 8.35

Disc Area. At T=0 the area of tissue discs used was 490.94 mm^2 . The greatest increase in area occurs when N and P are supplied at maximum concentrations and this

area is significantly greater than 0.5 N : 2.5 P ($P < 0.10$) and 0.5 N : 4.5 P ($P < 0.01$). However, the difference between all treatments (except 0.5 N : 4.5 P) is not statistically significant (Table 6 vi).

Therefore, disc area increase and dry weight change is greatest when nitrate and phosphate are supplied at maximum concentrations, but high phosphate concentrations are inhibitory when nitrate is at minimum concentrations (ie treatments of 0.5 N : 2.5 P and 0.5 N : 4.5 P).

Table 6 vii. a Total N content ($\mu\text{g N.100 mg dry wt}^{-1}$), b Internal P content ($\mu\text{g P.100 mg dry wt}^{-1}$) and c mannitol content (% dry wt) of discs of L. digitata grown for 20 days on a range of nitrate and phosphate concentrations in September at 14.75°C and with a photoperiod of 14 hrs L.

		$\text{NO}_3\text{-N } (\mu\text{g-at N.l}^{-1})$					
		0.5		5.5		10.5	
$\text{PO}_4\text{-P } (\mu\text{g-at P.l}^{-1})$	a	331.41	± 13.79	447.74	± 18.80	507.78	± 12.32
	0.5 b	14.32	± 1.02	15.03	± 0.82	19.95	± 3.00
	c	16.97	± 1.06	12.46	± 1.59	13.75	± 0.81
	a	319.70	± 20.44	421.13	± 24.04	491.23	± 37.49
	2.5 b	124.61	± 1.71	129.72	± 0.40	126.85	± 2.06
	c	16.99	± 1.35	10.96	± 1.37	11.53	± 1.55
	a	348.92	± 22.19	405.78	± 10.39	530.19	± 20.93
	4.5 b	225.43	± 6.50	230.48	± 6.23	243.65	± 13.30
	c	16.67	± 1.37	12.26	± 1.09	10.13	± 0.62

Internal Reserves (Table 6 vii)

Internal N (Total) Content. At $T=0$, the total N content of the tissue was $336.67 \pm 12.94 \mu\text{g N.100 mg dry wt}^{-1}$. At low external N concentrations ($0.5 \mu\text{g-at N.l}^{-1}$) the N content relative to the dry weight does not change greatly over the course of the experiment, but the change represents a mean actual increase of 4.6% of N by the discs over 20 days. At intermediate (5.5) and maximum ($10.5 \mu\text{g-at N.l}^{-1}$) N concentrations there is a mean increase of 33% and 64.8% respectively in the total (actual) amount of N in the discs which is recorded as an increase of 26.2% and 51.4% respectively in total N content relative to dry weight. Thus higher external N appears to accelerate photosynthesis more than N assimilation. The external phosphate concentration does not significantly affect tissue N content at the P concentrations tested.

Internal P content. At $T=0$ the relative P content of the discs was $35.93 \pm 5.86 \mu\text{g P.100 mg dry wt}^{-1}$. At minimum external P concentrations the relative P content drops to 54.3% of the original content which represents an actual loss of P from the tissue of $171 \pm 20 \mu\text{g P}$. The P is presumably lost by leakage to the medium although evidence from other workers suggests that this is likely to be low (see discussion). At $2.5 \mu\text{g-at P.l}^{-1}$ external concentration the relative P content and the sum total P in the discs increases (253.6% increase relative to the dry weight which represents an actual increase of $927.1 \pm 20.2 \mu\text{g P}$) and at maximal external P, internal P

increases by 549.2% with an actual increase of $1989.85 \pm 67.30 \mu\text{g P}$. The tissue P content is not significantly affected by the external nitrate concentration. At intermediate P and maximum P there is a mean increase of 186.1% and 527.4% respectively in the total (actual) amount of P in the discs which is recorded as an increase of 253.6% and 549.3% respectively in total P content relative to dry weight. Thus high external P concentrations accelerate P assimilation (particularly at $2.5 \mu\text{g-at P.l}^{-1}$) more than photosynthesis.

If the ratio of external concentration at maximum N+P and minimum N+P is compared with the tissue N and P content the internal reserves are lower than is predicted from the supply concentration - the ratio of supply concentration = 15 : 1, whereas that of the internal N+P content is only 2.24 : 1. Increase in area is also lower than predicted in the same treatments, 1.02 : 1. Hence the growth potential of L. digitata is not realised in September. The low internal N and P content may possibly be interpreted as the diversion of cell resources in the meristem from uptake to growth (see Chapter 3) and that although, by late summer growth potential is not realised as senescence effects become more important, senescence may also prevent the efficient division of resources into uptake.

Mannitol Content. At T=0 the mannitol content is $8.15 \pm 0.24\%$ of the dry weight, but this increases in all

treatments over the course of the experiment. The mannitol content is not significantly different between all treatments supplied with 5.5 and 10.5 $\mu\text{g-at N.l}^{-1}$ external concentration and shows a mean increase of 45.4%. However, low external nitrate results in a mannitol content which is significantly greater than that at high or intermediate N concentrations and over 20 days there is an increase of 107.2%. The mannitol content is unaffected by external phosphate concentration but minimal N results in a significantly increased mannitol content. This increased mannitol content of N-limited discs may be explained in terms of the low tissue N content limiting protein synthesis which in turn limits growth, hence carbohydrate utilisation is curtailed and mannitol accumulates (see Chapter 7).

Summary of Experiment. Dry weight and disc area increases are greatest when maximum concentrations of both nitrate (10.5 $\mu\text{g-at N.l}^{-1}$) and phosphate (4.5 $\mu\text{g-at P.l}^{-1}$) are supplied, although there is little difference between all treatments investigated. Intermediate and high external N and P concentrations did not result in rapid growth despite increasing internal N and P reserves and growth (area increase) was lower than predicted from the supply concentrations of N and P. It, therefore, appears that by September the tissue has, to a large degree, lost its ability for cell division and cell enlargement (ie the tissue has become senescent); there is a limited response

when N and P are supplied at or above $5.5 \mu\text{g-at N.l}^{-1}$ and $2.5 \mu\text{g-at P.l}^{-1}$ which may correspond to the transient increase in growth in situ in late September as seawater N and P increase.

It therefore, appears that senescence (loss of growth potential) is a long-term effect of nutrient depletion in Laminaria which is detected to a small degree in June/July but is very apparent by September.

However, extrapolation from disc growth experiments to whole plant growth causes a number of problems and there are disadvantages in using discs rather than intact plants. There may be growth promoting/influencing substances which, in the intact plant, are translocated either from the holdfast and stipe (there is little evidence of export in this direction) or from the distal frond tissue, and these would, therefore, be lacking to the discs. Wounding the tissue when the disc is cut may stimulate cell division around the cut edges to heal the wound. This may result in a small increase in disc area which may be interpreted as growth of that disc. In the field, holes punched in the meristem to measure in situ growth, form flaps of tissue along the cut edges, presumably by rapid cell division to prevent leakage of metabolites and the possibility of extensive bacterial invasion. It would be interesting to investigate the wound healing response from an histological aspect because the flaps develop and wounds are healed in frond tissue some distance

from the meristem. The cells in this region are no longer actively dividing or enlarging under normal circumstances yet wounding will initiate cell division.

In the disc growth experiments, there was no evidence of tissue flaps being formed (probably because of the short duration of the experiments - usually 20 days) and the increase in disc area by this increased cell division through wounding may be assumed to be minimal and will occur in all the discs. Large differences in disc area will result from growth from the experimental treatments.

Another disadvantage in using discs of tissue is the wide variation in growth under all treatments and discs cut from frond tissue distal to approximately 10 cm from the transition zone do not show any increase in area even during the Spring with supplementary N and P.

However, the advantages is using discs possible outweighs the disadvantages in that a large number of discs may be used per treatment (usually 15 per 2 l flask) and a large number of different plants are used as the source material (a larger sample size than is usually possible for whole plant experiments). Alternatively, in smaller experiments genotypic variation may be eliminated by using discs cut from a single plant.

The absence of a holdfast in the discs enables easier cleaning and removal of epiphytes than the intact plants and the limited internal reserves (N, P and carbohydrates) of the discs do not obscure changes in growth caused by

the experimental treatments. Also, in the cultures (2 l boiling flask) the tissue discs move around freely with the water current, the resultant narrowing of the boundary layer will enhance nutrient uptake and gaseous exchange in the discs more so than in the whole plant growth tubes.

But primarily there is no translocatory loss to another zone nor gain from it, therefore, discs are essential to distinguish local effects or responses from local + wider effects due to translocation of N, P and carbohydrates.

DISCUSSION

It has been suggested, on the basis of the rapid depletion of internal N and P reserves, that the decline in linear growth rates in May is a direct effect of the depletion of seawater N and P one month previously and that summer concentrations of nitrogen and phosphate are likely to limit in situ growth of L. digitata and L. saccharina. Enrichment experiments presented here (6 i(a-b), 6 ii(a), 6 iii(a)) indicate that both these suppositions are true.

During enrichment experiments undertaken in the Spring, growth rates of plants with added N and P initially increased rapidly but levelled off to a constant rate after about 12 days enrichment. Chapman & Craigie (1977) showed a similar levelling off in growth of L. longicruris enriched in situ with NO_3 . If N and P are the only

constraints on growth during the summer, this rate may indicate the maximum growth rate possible ie. the growth saturation rate at the given conditions, where uptake of N and P must directly supply all N and P growth requirements, since a deficit between uptake and growth requirements would result in utilisation of internal reserves and a corresponding growth rate decline. Internal reserves were not measured in these experiments but inorganic-N accumulated in the tissue of nitrate-enriched L. longicruris (and declined in the low-N controls) (Chapman & Craigie, 1977) suggesting that in this alga approximately 0.8 cm.day^{-1} represents the growth saturation rate in situ in July-September with uptake exceeding immediate metabolic requirements. Further experiments are required to provide information on the concentrations at which nitrogen (as nitrate) and phosphate are saturating and become limiting to growth of L. digitata and L. saccharina sporophytes both in culture and in situ. The only work to be published to date is the effect of nitrate concentration on the growth of L. saccharina (Chapman, Markham & Luning, 1978). They were able to show a linear relationship between growth and nitrate concentration up to $2.26\text{--}4.53 \text{ } \mu\text{g-at N.l}^{-1}$ substrate concentration; below $2.25 \text{ } \mu\text{g-at N.l}^{-1}$ there was no significant formation of internal reserves, ie. nitrate concentrations below $2.25 \text{ } \mu\text{g-at N.l}^{-1}$ are limiting to growth, but above $4.52 \text{ } \mu\text{g-at N.l}^{-1}$ 'luxury' consumption was detected. Summer

seawater nitrate concentrations (Chapter 4) fall below this figure of $2.25 \mu\text{g-at N.l}^{-1}$ thus providing further support for the hypothesis that summer nutrient (N+P) concentrations limit Laminaria growth. However, this growth saturation concentration is very low compared to winter nitrate levels at St. Andrews and the authors do not specify at what time during the year these experiments were undertaken. Extrapolation from laboratory to field conditions is difficult; the flow-through culture chambers used by Chapman et al (1978) did not simulate patterns of water turbulence in the sea and since NO_3^- -N uptake is dependent on water velocity (see Wheeler (1977) in Macrocystis pyrifera) this must be a major drawback in relating these figures to plants growing in situ. For example, assuming that extrapolation between species is valid, these results appear to show that in St. Margaret's Bay, Nova Scotia, L. longicruris is always nitrate-limited since ambient concentrations in the kelp beds do not exceed $1.35\text{--}1.58 \mu\text{g-at N.l}^{-1}$ (Chapman & Craigie, 1977). This is almost certainly not the case since storage of internal NO_3^- occurs during the winter.

Enrichment with N and P in June/July enhanced growth of L. saccharina suggesting external nutrient limitation, yet enrichment in September had little effect on growth of L. digitata, nor on growth of L. saccharina and L. digitata enriched with N or P at this time (Chapters 4 and 5). This suggests that N and P depletion in the seawater or some other factor declining in parallel causes

senescence of the frond tissue. The effects of this depletion are seen almost immediately in a decline in photosynthetic capacity but over the longer term loss of cell division and growth potential are also recorded.

The immediate effect of nutrient depletion is the decline in photosynthetic capacity or potential. The term 'senescence' is used guardedly in this context since it implies a non-reversible fall in metabolism and results from Drew (pers comm.) suggest that both L. digitata and L. saccharina fronds recovered high photosynthetic potential in January before the sampling zone (the lower part of the digitations in L. digitata and 10-15 cm from the transition zone in L. saccharina) was replaced by new frond tissue. Both species showed highest photosynthetic capacity early in the year (February-April) followed by a rapid decline in May to June to a minimum in August and then a slow increase during the autumn. Unlike, L. digitata and L. saccharina, L. hyperborea showed only a slight recovery in the autumn before the photosynthetic capacity decreased in the Spring just before the old frond is cast.

This loss of photosynthetic potential has been shown more recently by Küppers & Weidner (1980) in L. hyperborea by measuring the activity of 2 enzymes of the Calvin cycle of photosynthesis (RUBPC (ribulose-1, 5-bisphosphate carboxylase) and GAPDH (NADP dependent)(glyceraldehyde-3-phosphate dehydrogenase (NADP dependent))). The standard enzyme activity (which is assumed to be proportional to

the amount of enzyme present and therefore, of photosynthetic potential) rises steeply to a spring maximum (February-May) and then decreases to a minimum in late summer.

It appears that this may then be a true senescence effect in L. hyperborea (as there is no recovery of photosynthetic potential) but not so in L. saccharina and L. digitata where a slow recovery occurs after the summer minimum.

Although the photosynthetic capacity declines after a Spring maximum, Küppers & Weidner (1980) found (after adjustments were made for seawater temperature) that highest metabolic activity of these enzymes occurred in August despite enzyme quantity being at a minimum at this time of the year. This agrees with maximum photosynthetic activity in midsummer in L. saccharina (Johnston et al, 1977), L. longicruris (Hatcher, Chapman & Mann, 1977) and L. hyperborea (Luning, 1971) although contrary to this, Drew (cited in Kain, Drew & Jupp, 1976) recorded very low gross photosynthetic rates in situ during the summer and highest rates in the spring (March-May).

Maximum enzyme amounts (other enzymes also show a Spring maximum; Küppers & Weidner, 1980) early in the year is advantageous since it enables Laminaria to establish relatively high metabolic rates at a time when temperature is probably limiting growth assuming that there are adequate endogenous reserves from January-April and

sufficient light from April onwards (after reserves are largely depleted) to support growth at this time.

The decline in photosynthetic capacity in L. digitata occurs because enzyme levels would be expected to be seriously reduced by tissue expansion once internal N and P reserves have been rapidly utilised after depletion of seawater N and P, and the minimum enzyme quantity (August (Küppers & Weidner, 1980)) occurs when N-limitation is at its most critical. With enrichment, this decline in enzyme levels is presumably prevented and hence photosynthetic capacity is maintained. Because of increased enzyme activity in summer, deleterious effects of the decline in photosynthetic capacity (ie. decline in enzyme amount) may only become apparent in the autumn as photoperiod and seawater temperature decrease. In L. digitata and L. saccharina where photosynthetic capacity shows a recovery in the autumn, this loss of enzyme amount in the Spring appears relatively unimportant. It is perhaps an advantage for Laminaria, once spring growth is established, not to have to use valuable internal N reserves for enzyme synthesis to maintain spring enzyme quantities at a time when seawater N and P depletion is critical.

Apart from this immediate effect of a reduction in photosynthetic capacity with nutrient depletion a loss of growth potential is recorded in June/July, when growth of L. saccharina enriched with both nitrate and phosphate is increased above the controls but the actual rates

expressed are less than is predicted from the external N and P concentrations and the corresponding internal N and P content. By September, L. digitata shows only a very limited response to increasing external N and P concentration and similarly, in situ the increase in growth rates is slight and transient as external and internal N and P increase. Prolonged external limitation (May-September) possibly raises the tissue C:N and C:P ratio's to levels where senescence or loss of cell division and enlargement occurs. These ratios were not measured directly but total ash (which may be considered equivalent to the C content of the tissue) increases during the summer (Black, 1948) while the N and P tissue content (both relative and absolute amounts) decrease; Mann (1972a) observed a correlation of higher C:N ratios during periods of decreased growth in L. digitata.

As is consistent with senescence, uptake capacity is retained and accumulation of N and P from the medium occurs (in enrichment experiments) but despite increasing internal N and P growth can no longer be "switched on" and growth rates do not rapidly increase. Since the mature frond tissue does not grow only the meristem determines growth and therefore, nutrient depletion must most affect the meristem preventing cell division and/or cell enlargement in this area of the frond. Further evidence for the cessation of cell division is provided by Kain (1976). She showed that early in the year (until May) frond growth

in L. hyperborea could be accounted for in terms of cell division exclusively until March and both division and expansion from March until May. From May onwards cell division appeared to have ceased and there was little further increase in frond area. However, frond growth in L. hyperborea, unlike L. saccharina and L. digitata almost completely ceases after June (total increase in length during the slow growth period amounts to less than 5 cm; Kain 1976) the latter 2 species continue growing at a low rate and "switching off" of cell division may not be complete.

The evidence suggests that nutrient depletion causes a slowing of growth rates by "switching off" (to a large degree) cell division and cell enlargement in the meristematic zone; the reduction of growth potential becomes evident by June/July but is almost complete by September. This senescence effect is exogenously induced since the decline in spring growth is prevented, or at least delayed by maintaining winter N and P concentrations. However, if senescence is solely a nutrient depletion effect, senescence or loss of growth potential would not be apparent over the long-term and the fronds would have recovered the spring growth potential in the autumn as seawater N and P increase to winter concentrations. Yet it is in the autumn that the tissue is senescent and unable to respond, to any great extent, to the increasing seawater and tissue N and P content. Some other factor(s) must

be involved which, once triggered by nutrient depletion in the Spring become more important as the summer progresses. This phenomenon might be a programmed senescence to do with age or photoperiod, or in fact dormancy.

The role of photoperiod has not here been studied but it might be important since "senescence" is shown here to be an effect of mid-late summer only; and rejuvenation of meristematic activity in January is associated with increasing daylengths despite low temperature rate-limitation of growth and no great change in external and internal nutrient levels. This could best be tested by experimentally lengthening the photoperiod in late summer and autumn in conjunction with adequate levels of N and P to investigate whether the decline in growth rates could be prevented at this time. The effect of increased photoperiod on growth of L. saccharina in November was investigated (Chapter 3) and although growth rates increased with increasing photoperiod up to 15 hours light the difference between all treatments investigated (photoperiods 6, 9, 12, 15, 18 hours light) was not significant. L. saccharina did not show a significant increase in growth rates as might have been expected if photoperiod was the principle factor preventing cell division and cell enlargement at this time. However, further experiments are required to investigate the role of photoperiod in the late summer and autumn (August/September/October) since decreasing daylength may possibly

have had an effect of initiating senescence or dormancy earlier in the season. But there is clearly a senescence/dormancy effect occurring at this time of year since there is a qualitative change in the algal response to light between November and December. As stated above, increased photoperiod in November resulted in growth rates not significantly greater than growth in the short day controls whilst increased photoperiod in December resulted in a dramatic growth rate increase by 'switching on' cell division whereas growth previous to this was mainly by cell enlargement (Chapter 3). Further information is required to establish whether the loss of growth potential can be explained in terms of photoperiod or whether this senescence effect is a genetically programmed phenomenon. It could be the effect of unrepressed genes limited by shortages of substances (possibly N and/or P) for enzyme synthesis or senescence operating through a system of active versus repressed dormant genes. Further research is required on this aspect of the biochemical control of growth in Laminaria before the possible factors involved can be established.

Tissue maturation with loss of meristematic activity is a normal feature of Laminarian morphogenesis in cells distal to the meristematic zone. Therefore, there is a senescence effect with distance from the meristem and hence with tissue age, as well as a possible senescence effect with season.

Once growth becomes limited by nutrient depletion

in the Spring, the area of the active meristem will decrease during the summer as the lower growth rates mean that displacement of tissue away from the meristem is reduced. Therefore, tissue within the basal 10 cm of the frond (the meristem) in, for example, September is older than tissue in the basal 10 cm of the frond earlier in the year (eg April) when maximum growth rates result in a rapid displacement of tissue distally. This would have the effect of a basally spreading wave of tissue maturation, which, perhaps by mid-late summer has spread through almost the entire meristematic zone. The reduced response to increased nutrients may simply reflect the significantly reduced meristematic area available to respond to increased nutrient conditions. However, such as effect alone cannot explain the changing response of Laminaria to light prior to new frond growth in January.

Nutrient depletion, therefore, appears important in both senescence with season and senescence with distance along the frond (once growth is limited by nutrients from April onwards). But nutrient depletion is unlikely to be the sole factor involved in senescence in Laminaria and particularly during the second half of the year other exogenous (eg Photoperiod) or endogenous controls become more prominent.

To summarise; the decline in seawater N and P is directly causal to the decline in spring growth rates in L. saccharina and L. digitata, and summer N and P

concentrations are limiting in situ growth. Prolonged nutrient depletion induces senescence of the frond tissue by reducing cell division and cell enlargement; the early effects of this senescence are seen by an immediate reduction in photosynthetic capacity and by June/July as a reduction in growth potential of L. saccharina. By September cell division has almost completely ceased, the tissue only shows a limited growth response to high external N and P concentrations while uptake and accumulation of N and P is maintained.

Nutrient depletion is responsible for senescence of the frond tissue although other exogenous and endogenous controls probably become more important towards the end of the year.

CHAPTER 7

ENDOGENOUS CARBOHYDRATES AND GROWTH

INTRODUCTION

The carbohydrates of the Phaeophyta and in particular the Laminariales have been extensively investigated from a commercial aspect. As a result fluctuations in carbohydrate content have been considered in relation to

i Season

(Black, 1948, 1950; Haug & Jensen, 1954; Jensen & Haug, 1956; Munda, 1962; Powell & Meeuse, 1964; Johnston, Jones & Hunt, 1977; Yokoyama et al, 1980)

ii Exposure

(Black, 1948, 1950)

iii Depth

(Black, 1948, 1950; Chapman & Craigie, 1978; Luning, 1979)

iv Geographical latitude

(Haug & Jensen, 1954)

v Chemical Composition of the seawater

(Black & Dewar, 1949)

In any growth study, endogenous carbohydrates must be considered since the growth rate is affected by external factors acting both directly, through cell division and cell enlargement and indirectly through carbohydrate reserves (energy and structural reserves

for later use). The three principal carbohydrates in the brown algae are alginic acid (a complex polyuronide), mannitol (polyhydroxy alcohol) and laminarin (β -1, 3-linked glucose polymer). The former, quantitatively the most important occurs intercellularly and in the cell walls where it has both structural and ion exchange properties (Percival, 1979). Once formed it is metabolically inaccessible, and thus it is the soluble intracellular carbohydrates, mannitol and laminarin which represent readily utilisable energy reserves.

Seasonal variation of mannitol and laminarin content in species of Laminaria is well known (see above). However, seasonal measurements are undertaken again in this study for the 2 reasons

- i that carbohydrate content varies with exposure, depth and chemical composition of the seawater, and
- ii because most of the British Laminaria studies have been carried out on the West Coast of Scotland where conditions differ from these East coast sites.

It is necessary to have details of the carbohydrate content of the algae from the actual areas where growth and nutrient measurements have been made in case of carbohydrate limitation of growth and to provide as near complete picture as possible of the growth conditions of L. saccharina and L. digitata.

Seasonal levels of mannitol and laminarin are investigated to ascertain

- i whether carbohydrate reserves are adequate for growth demands throughout the year or whether carbohydrates become limiting and hence effect a change in growth rate, and
- ii whether growth rate is directly related to carbohydrate content and hence, can differences in growth rates between the sampling sites be attributed to differences in endogenous carbohydrate levels.

Circumstantial evidence suggests that carbohydrates are unlikely to be limiting growth at any point during the year since they would be expected to be at their most limiting during the winter (period of minimum light (photoperiod and irradiance) and minimum seawater temperature), yet it is at this time that rapid spring growth is initiated and apparently supported by available carbohydrate reserves.

The relationship between carbohydrate content and the external variables - daylength, seawater temperature and $\text{NO}_3\text{-N}$ concentration are considered and discussed in relation to seasonal growth of L. digitata and L. saccharina.

Alginic acid also varied seasonally in Laminaria but due to its metabolic inaccessibility it is regarded rather as a sideline where growth is considered and as a

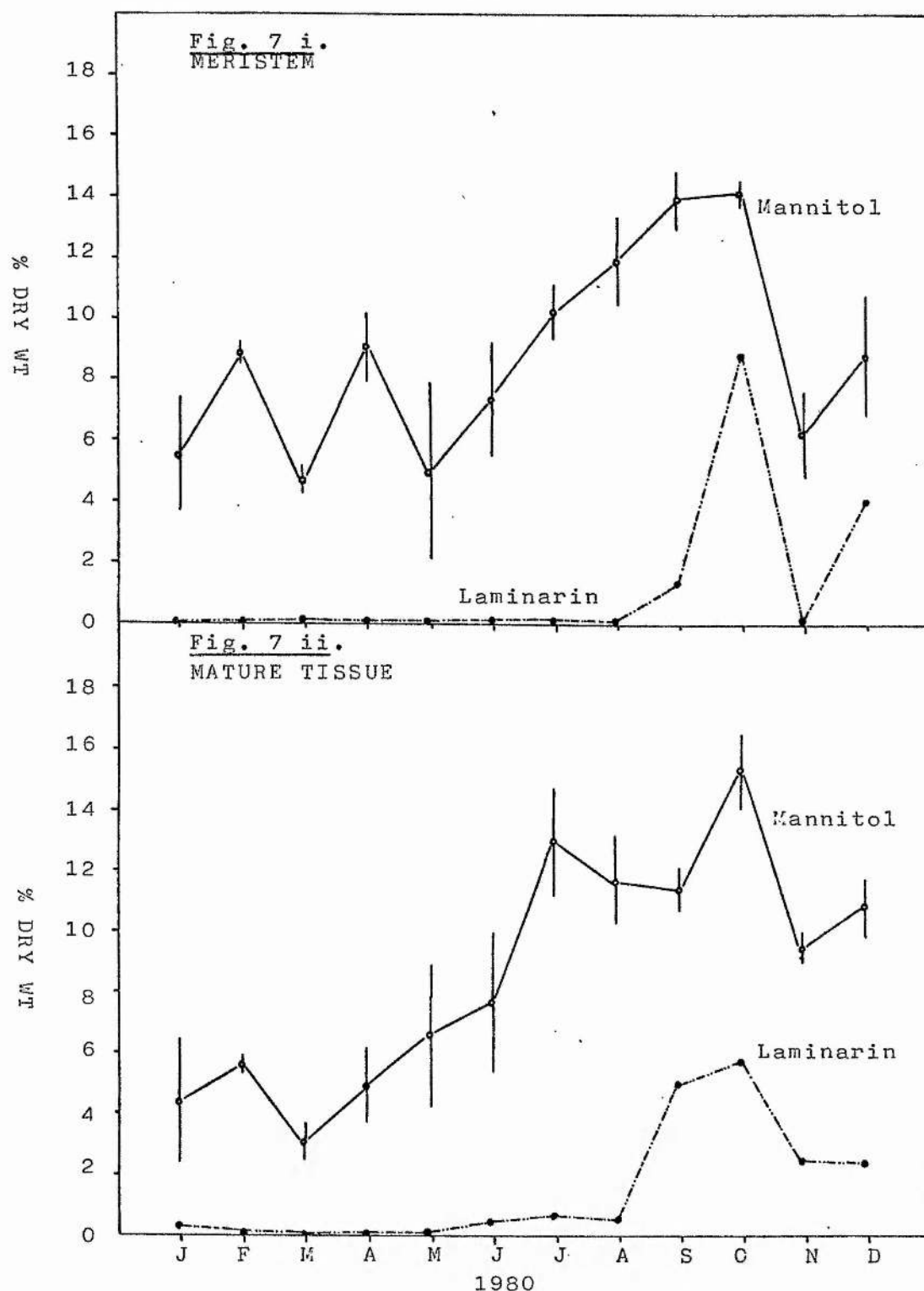


Fig. 7 i. Mannitol and laminarin content of the meristem of *L. saccharina* at St. Andrews during 1980.

Fig. 7 ii. Mannitol and laminarin content of the mature frond tissue of *L. saccharina* at St. Andrews during 1980.

(Figures are MEAN \pm SE with 3 replicates/treatment)

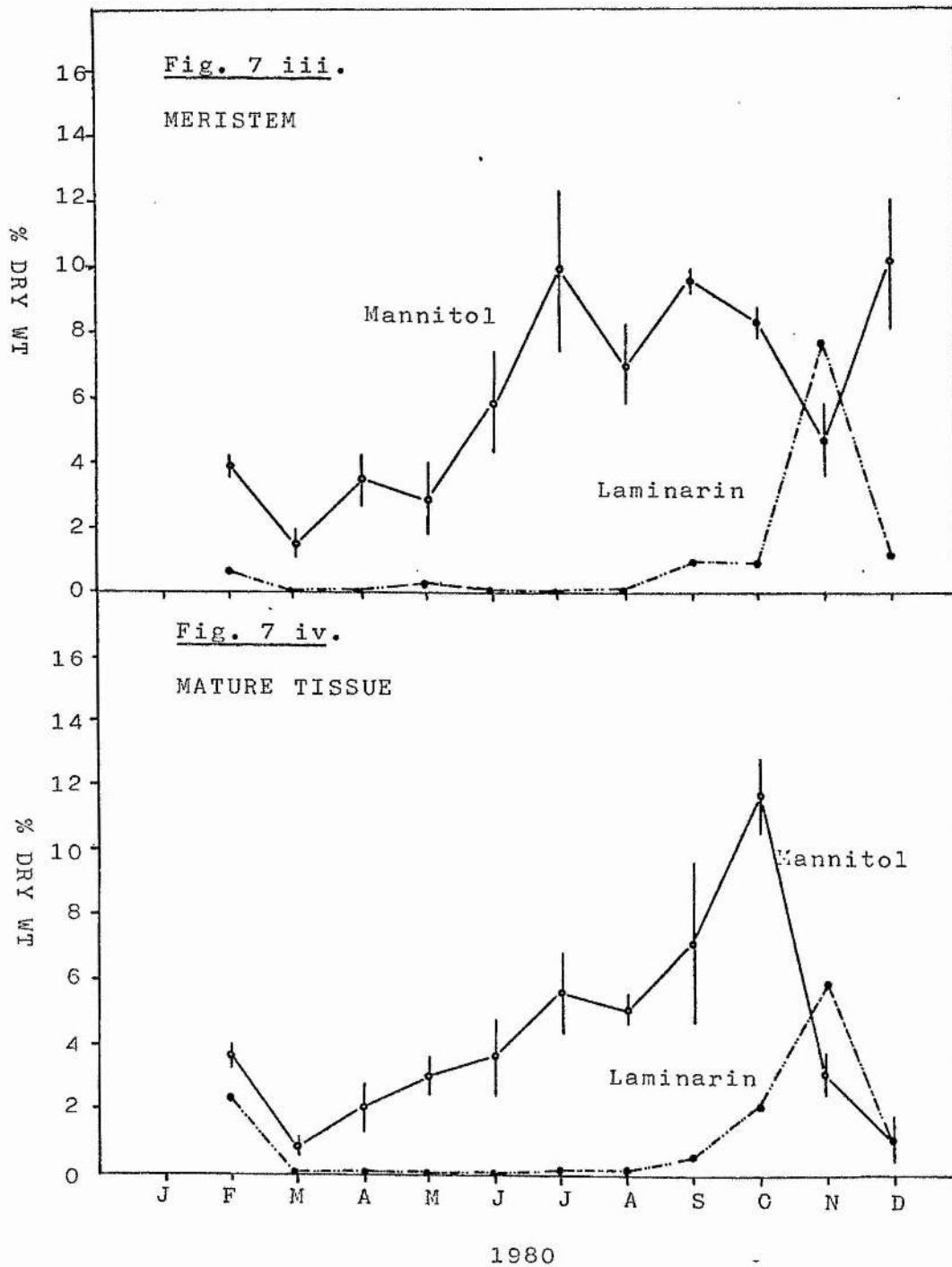


Fig. 7 iii. Mannitol and laminarin content of the meristem of *L. saccharina* at St. Andrews Sewer during 1980.

Fig. 7 iv. Mannitol and laminarin content of the mature frond tissue of *L. saccharina* at St. Andrews Sewer during 1980.

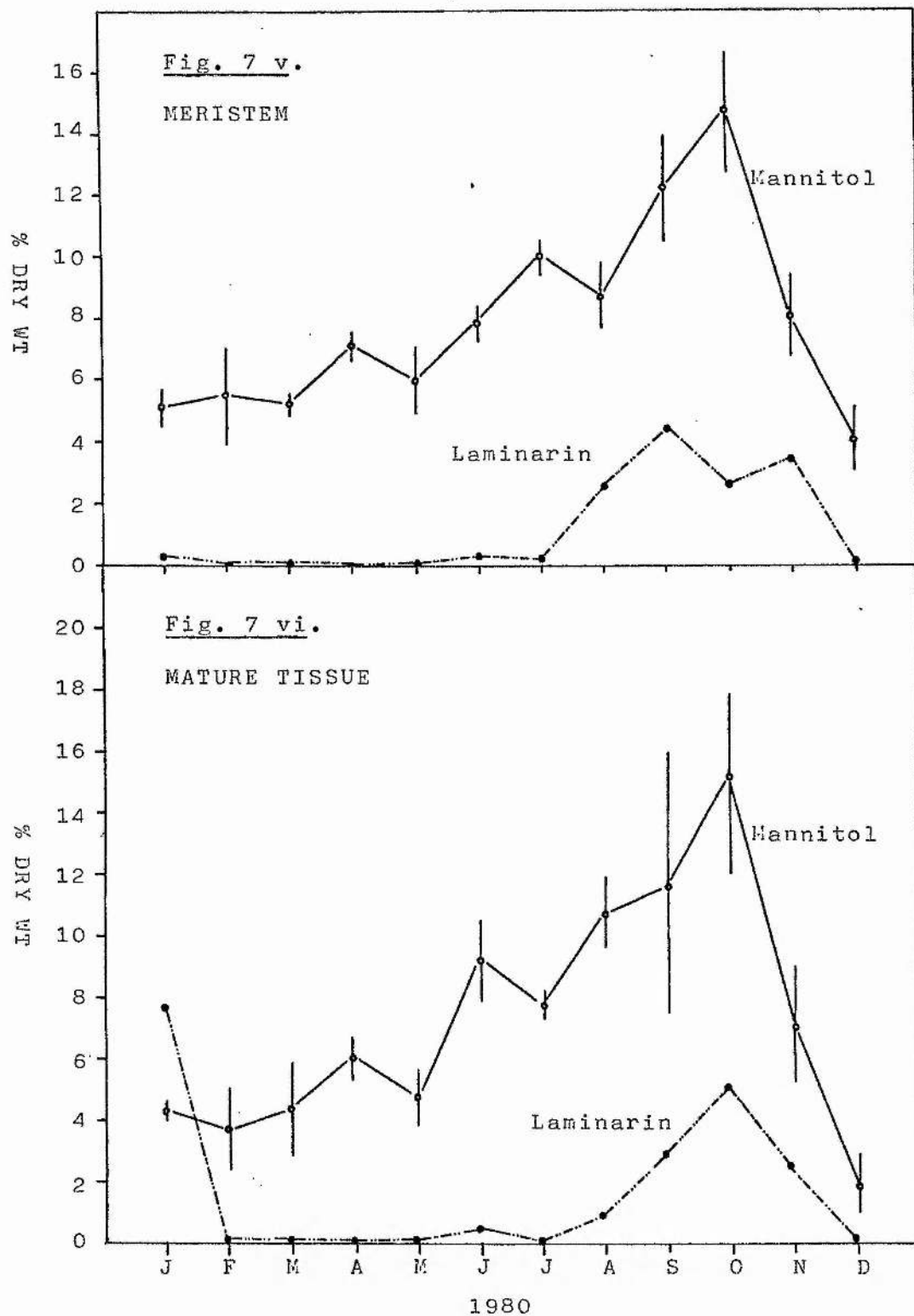


Fig. 7 v. Mannitol and laminarin content of the meristem of *L. saccharina* at Kingsbarns during 1980.

Fig. 7 vi. Mannitol and laminarin content of the mature frond tissue of *L. saccharina* at Kingsbarns during 1980.

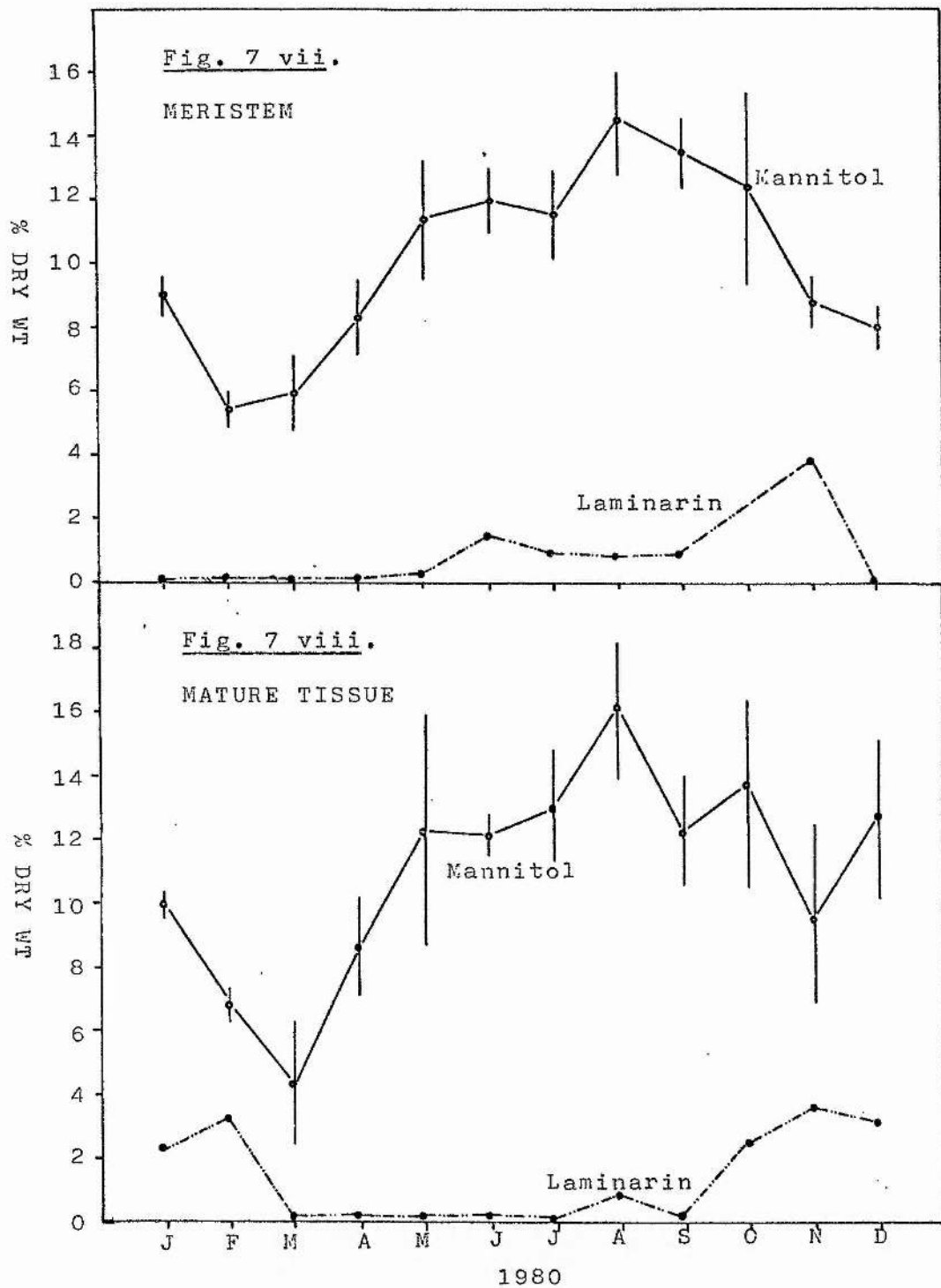


Fig. 7 vii. Mannitol and laminarin content of the meristem of L. saccharina at Fifeness during 1980.

Fig. 7 viii. Mannitol and laminarin content of the mature frond tissue of L. saccharina at Fifeness during 1980.

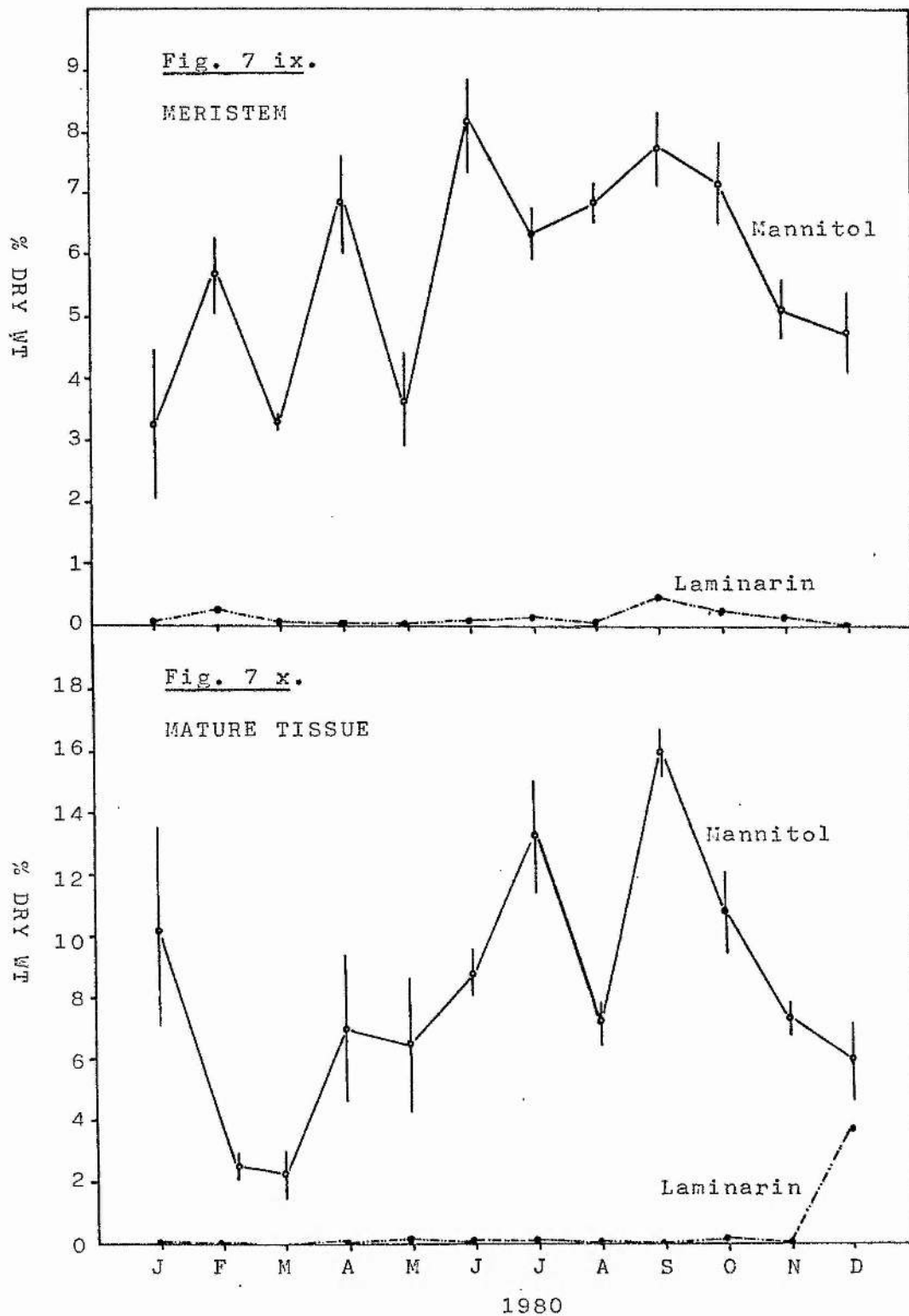


Fig. 7 ix. Mannitol and laminarin content of the meristem of *L. digitata* at St. Andrews during 1980.

Fig. 7 x. Mannitol and laminarin content of the mature frond tissue of *L. digitata* at St. Andrews during 1980.

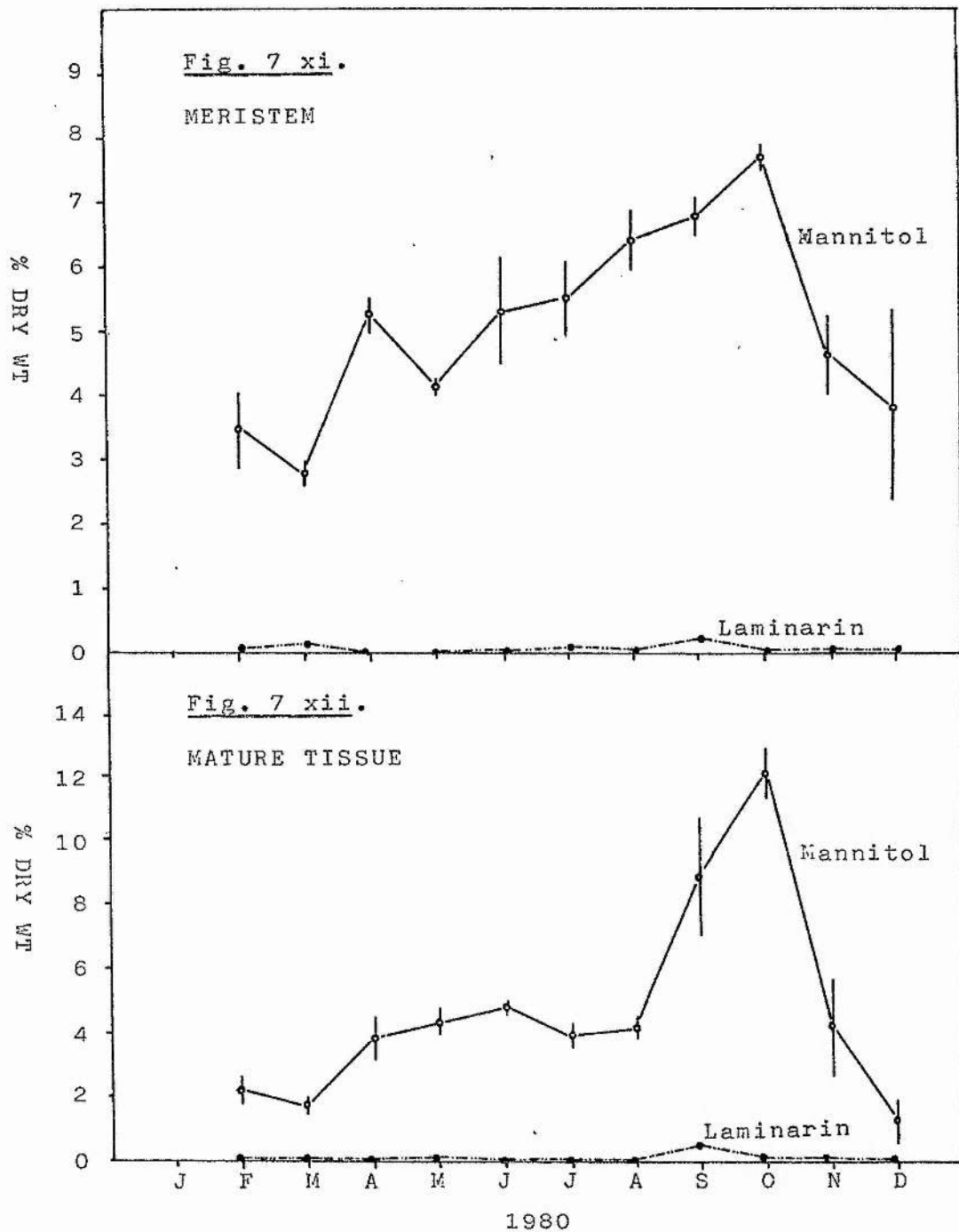


Fig. 7 xi. Mannitol and laminarin content of the meristem of L. digitata at St. Andrews Sewer during 1980.

Fig. 7 xii. Mannitol and laminarin content of the mature frond tissue of L. digitata at St. Andrews Sewer during 1980.

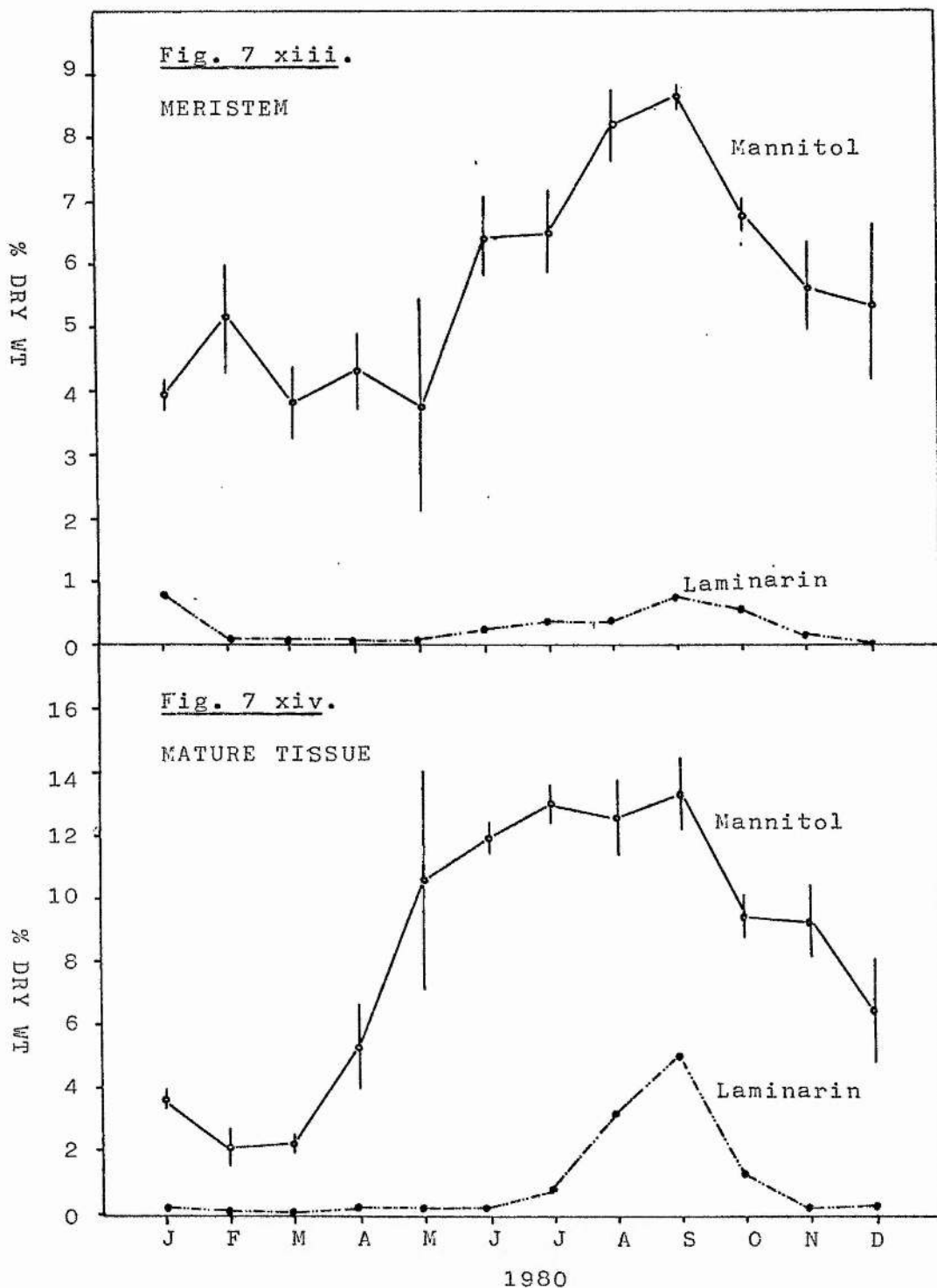


Fig. 7 xiii. Mannitol and laminarin content of the meristem of L. digitata at Kingsbarns during 1980.

Fig. 7 xiv. Mannitol and laminarin content of the mature frond tissue of L. digitata at Kingsbarns during 1980.

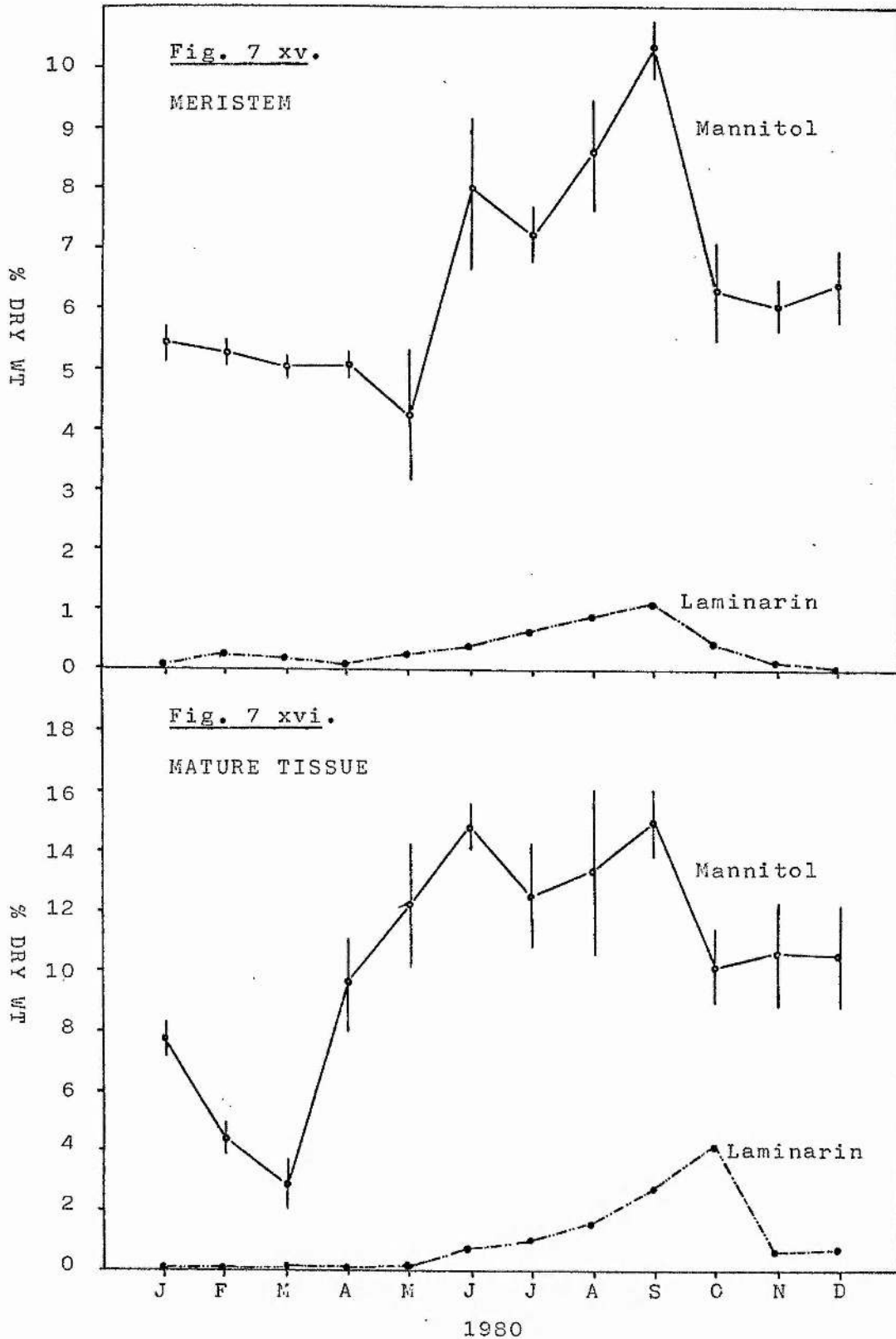


Fig. 7 xv. Mannitol and laminarin content of the meristem of *L. digitata* at Fifeness during 1980.

Fig. 7 xvi. Mannitol and laminarin content of the mature frond tissue of *L. digitata* at Fifeness during 1980.

result has been investigated in less detail than either mannitol or laminarin.

RESULTS

Both L. saccharina and L. digitata show a similar seasonal pattern in mannitol and laminarin content (on a dry weight basis, ie proportional), at the 4 sampling sites (Figs 7 i-xvi).

i MANNITOL

After a minimum content in March, mannitol accumulates during the summer to a peak in June/July, falls slightly in August before increasing to a maximum in September/October.

a) Variation in mannitol content between the meristem (2 cm) and the more distal tissue (20 cm)

L. saccharina. The mannitol content is similar in both the meristem and the distal tissue during the autumn maximum (October) and the Spring minimum (March). There are 2 exceptions to this, at St. Andrews sewer (Figs 7 iii and 7 iv) where mannitol content is significantly greater at 20 cm than at 2 cm ($P < 0.10$) in October, and at St. Andrews (Figs 7 i and 7 ii) where the meristem had a greater percentage of the dry weight as mannitol than the distal tissue ($P < 0.10$) in March.

L. digitata. In L. digitata there is a significant difference between the 2 tissue areas during both maximum and minimum mannitol content.

At maximum content (Sept/Oct) mannitol was significantly higher in the distal than in the meristem tissue (St. Andrews $P < 0.001$; St. Andrews sewer $P < 0.01$; Fifeness $P < 0.02$; Kingsbarns $P < 0.02$). In March, the reverse occurs with the meristem containing more mannitol on a dry weight basis than the older tissue (St. Andrews NS; St. A Sewer $P < 0.05$; Fifeness $P < 0.10$; Kingsbarns $P < 0.05$).

b) Variation between Sites

L. saccharina. Plants from Fifeness, Kingsbarns and St. Andrews have a similar mannitol content at 20 cm along the frond with a mean value of 14.80 and 3.66% of the dry weight at maximum and minimum content respectively. Plants at the sewer site have significantly less mannitol at 20 cm than those at St. Andrews ($P < 0.10$) at maximum content and less than plants at Kingsbarns ($P < 0.10$) and St. Andrews ($P < 0.02$) at minimum content in February/March. In the meristem tissue (2 cm) plants at the sewer site have significantly reduced mannitol content compared to the other 3 sites (St. Andrews $P < 0.01$, $P < 0.01$; Kingsbarns $P < 0.01$, $P < 0.05$; Fifeness $P < 0.01$, $P < 0.05$) at minimum and maximum mannitol content respectively. St. Andrews, Kingsbarns and Fifeness show similar maximum and minimum tissue mannitol content.

L. digitata. The minimum mannitol content is similar at all 4 sites at 20 cm along the frond. Fifeness, Kingsbarns and St. Andrews show a similar maximum content but sewer site plants have a lower maximum content than

St. Andrews ($P < 0.02$), Fifeness ($P < 0.10$) and Kingsbarns (NS).

In the meristem, the minimum and maximum mannitol content recorded at the sewer site is lower than at the other 3 sites (St. Andrews, $P < 0.10$, NS; Fifeness $P < 0.002$, $P < 0.01$; Kingsbarns NS, $P < 0.05$; respectively). In September/October plants at Fifeness have a significantly higher mannitol content than sewer ($P < 0.01$), St. Andrews ($P < 0.05$) and Kingsbarns ($P < 0.02$).

ii LAMINARIN

The seasonal variation of laminarin in the frond shows very low levels from February until June/July, accumulating after this to a peak in late summer (Sept.-Nov.). (Figs 7 i-xvi)

Laminarin content is generally considerably less than that of mannitol both in the meristem and in the more distal tissue; L. saccharina at the sewer site in November (Fig. 7 iii and 7 iv) and Kingsbarns at 20 cm along the frond in January (Fig. 7 vi) are exceptions to this.

a) Variation in laminarin content between the meristem (2 cm) and the more distal frond tissue (20 cm)

L. saccharina. Despite wide variation, it is apparent that laminarin content of the meristem generally exceeds that of the distal tissue during the spring and summer whereas the reverse is true during the winter months. This trend is more clearly shown in L. digitata

where laminarin content of the meristem is greater than or equal to that of the more mature tissue from January to June and that from June/July until December distal tissue laminarin content exceeds that of the meristem.

From June onwards, laminarin production may be at a maximum, the slowing of Laminaria growth reduces the demand for carbohydrates and laminarin accumulates at the site of production, hence laminarin in the distal tissue exceeds that of the meristem. During the late winter and spring (January onwards) carbohydrate demand for growth is greater and laminarin accumulated in the distal tissue the previous autumn may be translocated basally to the meristem, hence laminarin content of the meristem is greater than that of the distal tissue. Low light may prevent further production of laminarin in the distal tissue at this time.

b) Variation between sites

L. saccharina. There is little definite pattern between laminarin content (at 2 and 20 cm along the frond) and the site of collection of the plants and throughout most of the year variation between the sites is not significantly different. Laminarin content peaks during different months at the various sites: in November at St. Andrews sewer and Fifeness, in October at St. Andrews and in January (at 20 cm) and September (at 2 cm) at Kingsbarns.

L. digitata. Like L. saccharina, laminarin content

of the frond is not significantly different between the 4 sampling sites, but St. Andrews sewer, with the exception of Jan-Mar (at 2 cm) and January and April (at 20 cm), tends to be lower than elsewhere. The maximum laminarin content of L. digitata occurs in different months at the different sites (Sewer and Kingsbarns in September, St. Andrews in December and Fifeness in October (20 cm) and September (2 cm), the timing of the peaks does not correspond with peaks in laminarin content in L. saccharina. It seems unlikely that laminarin is built up directly in response to external factors eg light and temperature, since these factors are similar between the sites and variation in exposure and nutrient concentration do not offer an adequate explanation. It is more likely that laminarin content is related to endogenous factors, eg internal N and P content or mannitol content as well as growth and respirational demands.

c) Relationship between Laminarin and Mannitol content

Laminarin content increases in the frond as mannitol accumulates during the summer. If laminarin and mannitol are interconverted and laminarin is synthesised once mannitol content has risen above a threshold value (as suggested by Black, 1948), then looking at the mannitol content of the frond in the month preceeding laminarin accumulation may give an indication of a threshold value (Table 7 i).

In L. digitata laminarin content begun to increase in the meristem after mannitol content had reached a mean value of 6.03% dry wt, whereas the mean mannitol content was higher in L. saccharina at 10.78% dry wt. The wide variation indicates that a threshold value may be unlikely although it is difficult to postulate when using data collected over a single year only.

Table 7 i: Mannitol content of the frond (% dry wt) in the month preceding laminarin accumulation. Mean \pm SE

SITE	MONTH	<u>L. saccharina</u>	<u>L. digitata</u>
		2 cm	2 cm
St. Andrews	Aug	11.84 \pm 1.42	6.82 \pm 0.33
Sewer	Aug	9.69 \pm 1.09	6.64 \pm 0.38
Fifeness	May	11.50 \pm 1.86	4.14 \pm 1.21
Kingsbarns	July	10.07 \pm 0.63	6.51 \pm 0.64

The results for the mature tissue (20 cm) are more variable; this variation may reflect the state of the tissue, which may possibly be undergoing senescence or the situation may be confused by the possibility of the translocation of carbohydrates basally.

d) Growth and Mannitol Content

The results suggest that during parts of the year growth rate and mannitol content may be correlated (Figs 7 xvii + xviii). From January/February to May

Fig. 7 xvii. Relationship between frond growth rate and the mannitol content of the meristem and mature tissue of L. saccharina at St. Andrews during 1980.

Fig. 7 xviii. Relationship between frond growth rate and the mannitol content of the meristem and mature tissue of L. digitata at St. Andrews during 1980.

Overpage:

Fig. 7 xix. Relationship between frond growth rate and the laminarin content of the meristem of L. saccharina at St. Andrews during 1980.

Fig. 7 xx. Relationship between frond growth rate and the laminarin content of the meristem of L. digitata at St. Andrews during 1980.

Fig. 7 xvii.

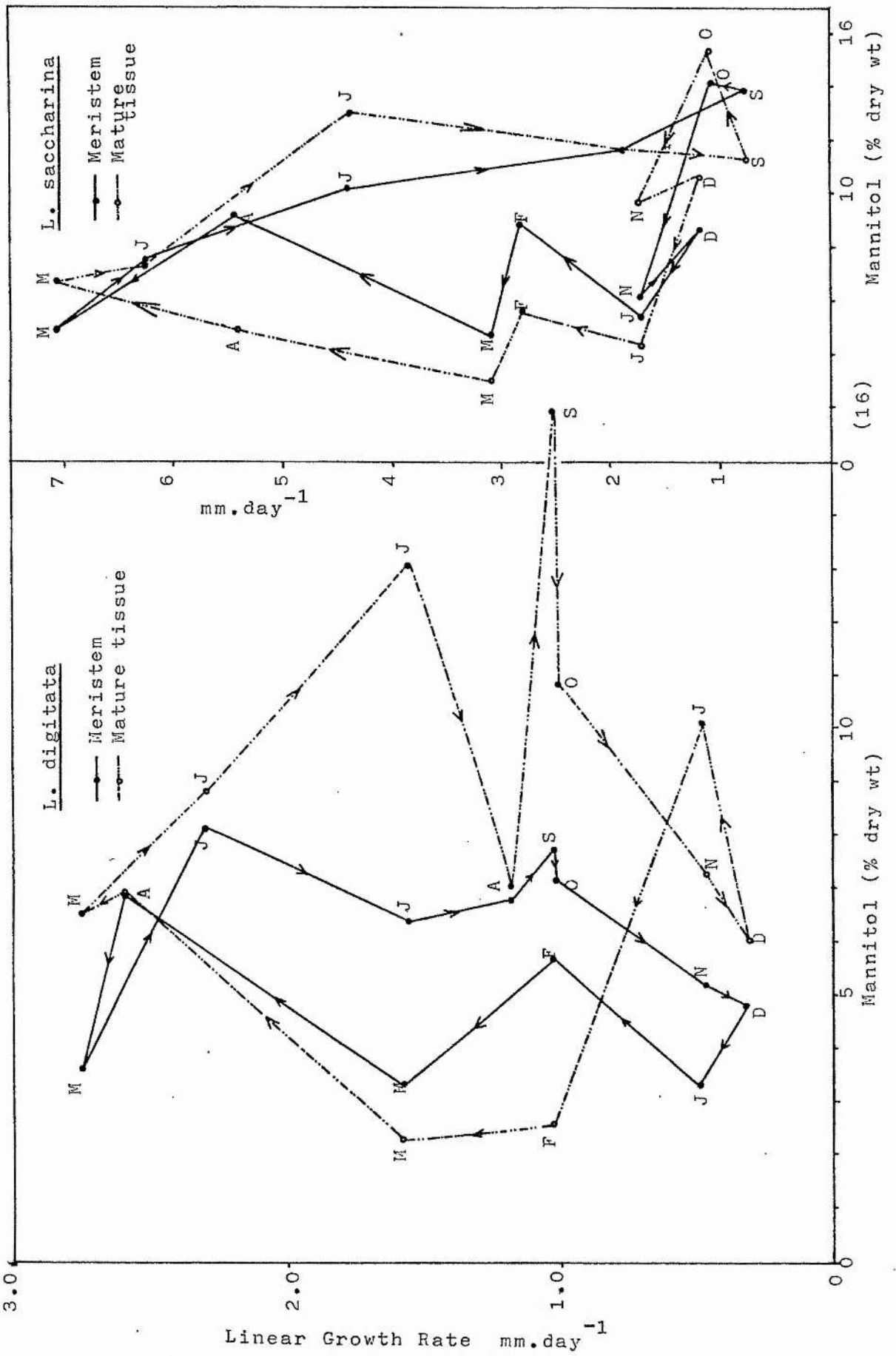
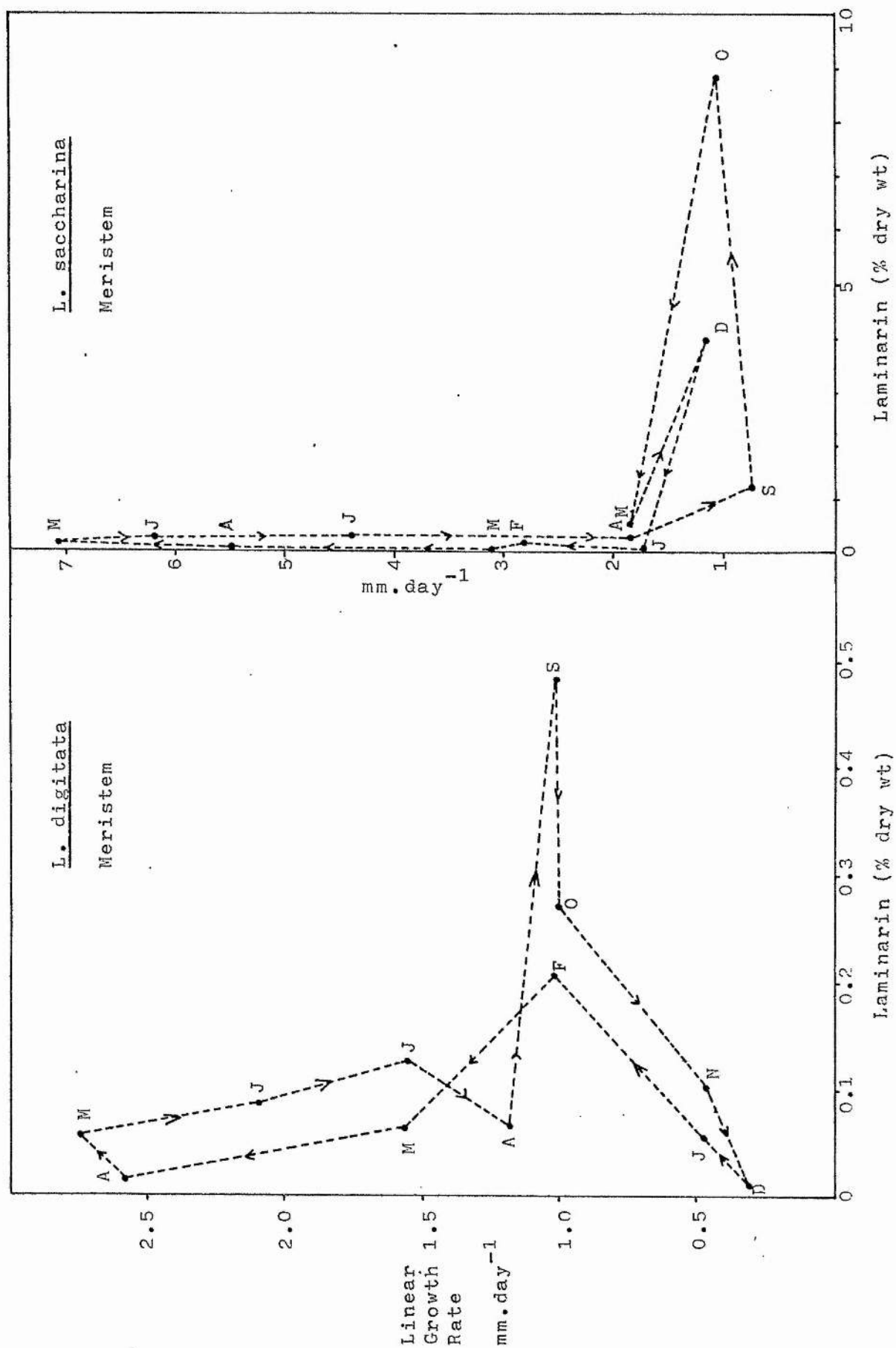


Fig. 7 xix.



growth increases as mannitol content increases in both the meristem and the more distal tissue in L. saccharina and L. digitata. From May to September growth decreases as mannitol increases and at the end of the year, from October onwards growth decreases as mannitol decreases (in L. digitata) but growth increases as mannitol decreases in L. saccharina. Growth rates begin to slow (in May) whilst mannitol content is still increasing. Mannitol content is then, unlikely to be causal in this decline. The summer increase in mannitol content may be due to the decreasing growth rate since the mannitol is being diluted by and utilised for growth to a lesser extent.

In L. digitata the declining growth rate from October-December may at least in part be brought about by the decreasing mannitol content. However, mannitol is unlikely to be actually limiting to growth at this time since rapid spring growth is initiated in January as mannitol content is still declining to the winter minimum. Mannitol content cannot therefore, be acting as the "trigger" to initiate this growth nor can it be limiting growth prior to or at this time since rates progressively increase. Even at the minimum content recorded at St. Andrews (4.75% and 3.27% dry wt in L. saccharina and L. digitata meristem respectively), there is still a significant quantity of mannitol available. Assuming that 80% of the tissue is water, then

4% dry wt is equivalent to 0.8% in the tissue total water, and assuming that half of the tissue water is cytoplasmic - 1.6% mannitol in the cytoplasmic water or 16 g mannitol/l. This gives the comparatively high minimum mannitol content of 89 mM.

Further support for the hypothesis that mannitol is never at levels limiting to growth is evident since high growth occurs at low mannitol in March and low growth occurs at high mannitol in October.

e) Growth and Laminarin Content

During most of the year (February-October) laminarin and growth of L. digitata (Fig. 7 xix) are inversely related, ie laminarin decreases as growth increases until May and increases as growth decreases to October. For the winter period, October-February a positive correlation is exhibited. Although laminarin decreases as growth declines it is unlikely that low levels of laminarin are causal to this decline since total soluble carbohydrate (mannitol + laminarin) is still relatively high at this time.

In L. saccharina laminarin and growth are similarly negatively correlated from January-September but in contrast to L. digitata they are also inversely related over the winter period (September-January). (Fig. 7 xx)

In both species, however, laminarin is present in very low amounts throughout most of the year and if carbohydrate reserves are important in controlling seasonal growth of Laminaria it is much more likely to

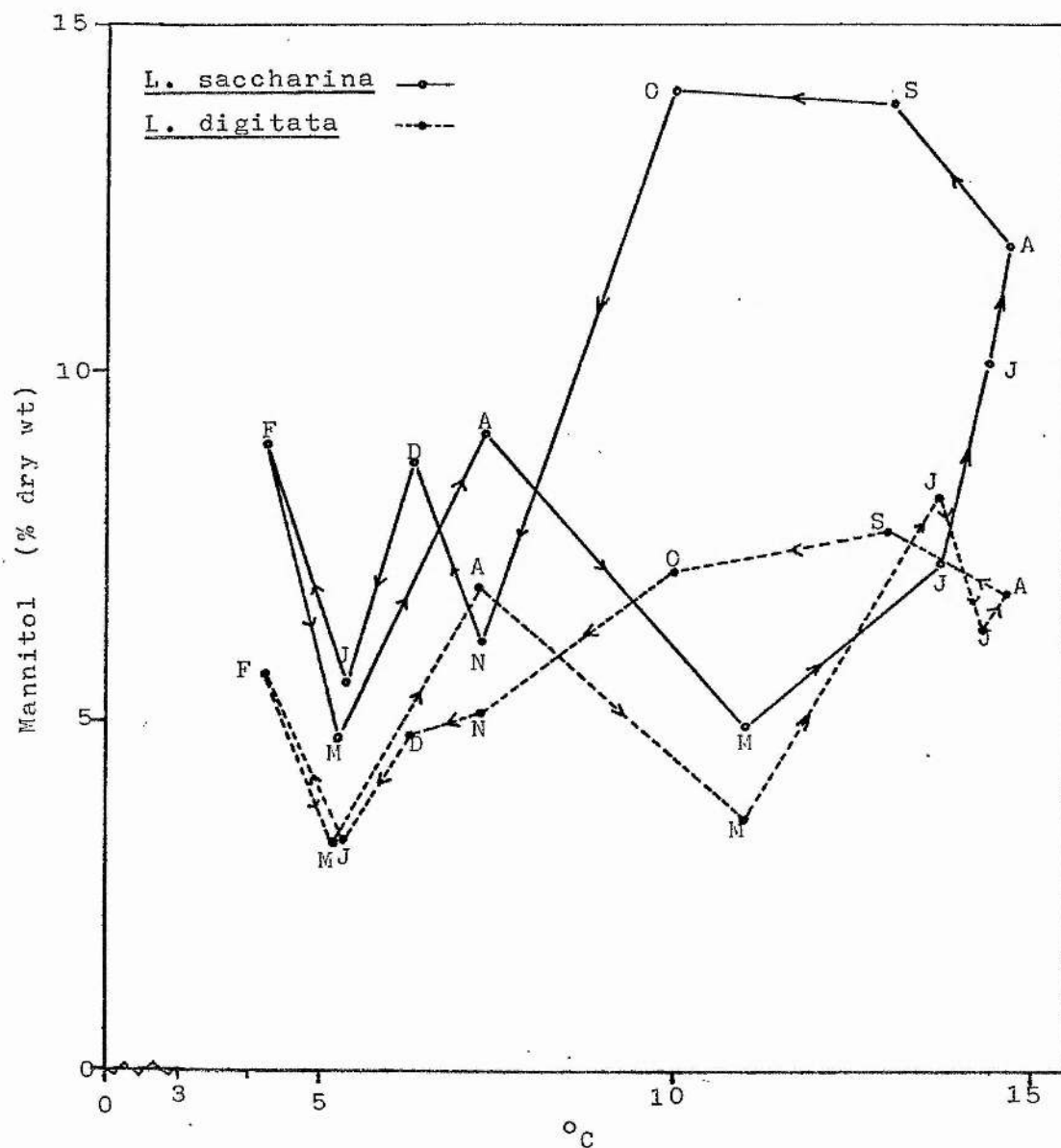


Fig. 7 xxi. Relationship between seawater temperature and mannitol content of the meristem of L. saccharina and L. digitata at St. Andrews during 1980.

be through the effects of mannitol than laminarin on the basis of the higher quantities involved and solubilities. Both laminarin and mannitol are largely vacuolar in occurrence and as a result are relatively inaccessible, but mannitol is more easily transportable because of its low molecular weight than the less soluble, high molecular weight laminarin.

f) Mannitol Content and Seawater Temperature

Mannitol content of the meristem of both L. digitata and L. saccharina shows an overall positive correlation with seawater temperature (Fig. 7 xxi). From November-April (L. saccharina) mannitol content fell as temperature declined. During the winter, at periods of comparable seawater temperatures, ie January and March, the mannitol content was similar suggesting a possible relationship between these 2 variables. However, after March, there was a wide discrepancy between mannitol content at comparable seawater temperatures - ie comparing April with November, May with October and June with September.

In L. digitata, mannitol content increases in a series of steps as temperature increases but from September to January these 2 factors show a significant linear correlation and decline in mannitol content may be partly attributed to decreasing seawater temperature.

g) Mannitol Content and Daylength (Fig. 7 xxii)

Daylength was measured as the number of hours of daylight on the 15th day of each month, estimated from

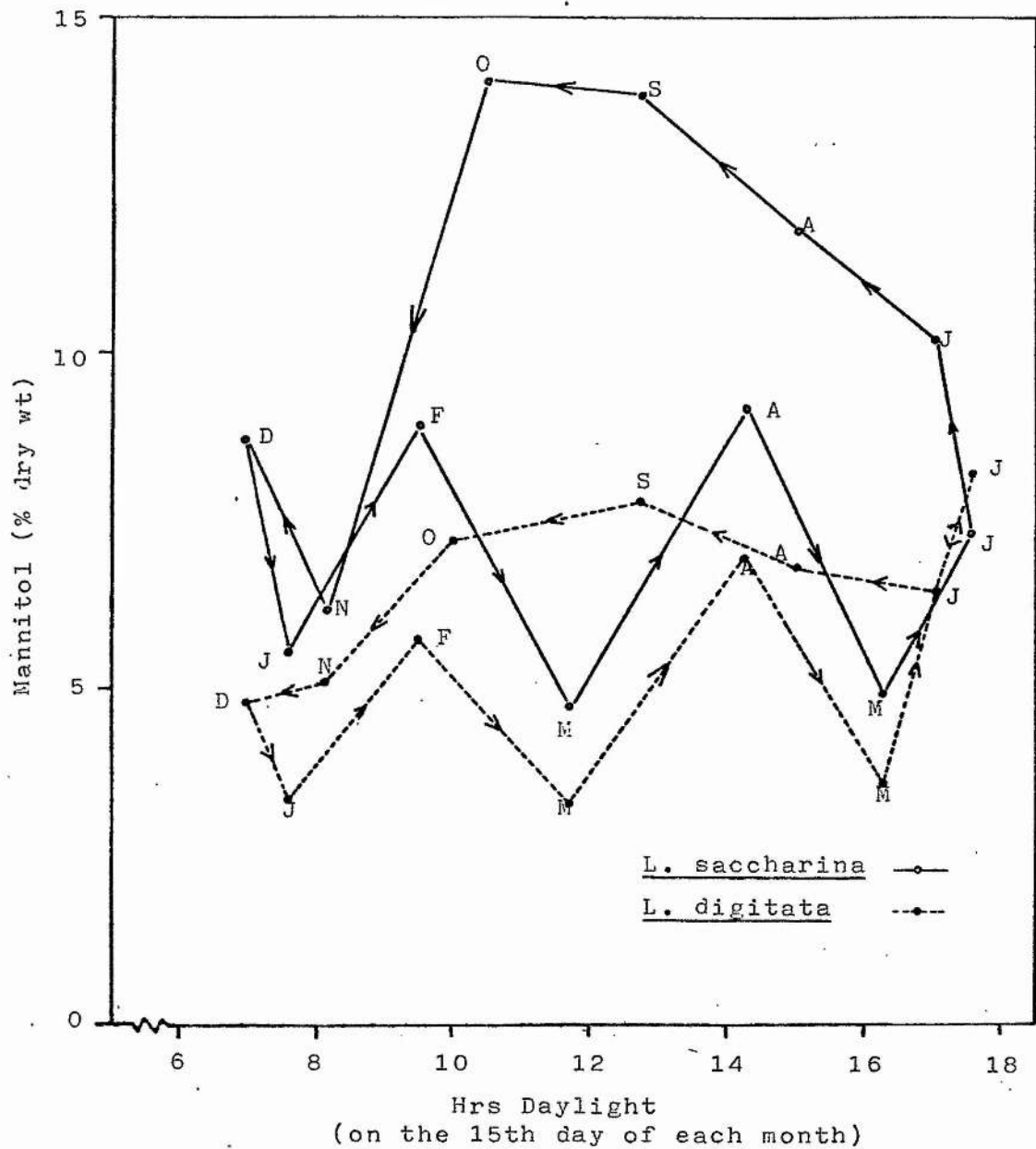


Fig. 7 xxii. Relationship between daylength and mannitol content of the meristem of *L. saccharina* and *L. digitata* at St. Andrews during 1980.

the Nautical Almanac 1980. During the first half of the year, mannitol content of L. saccharina and daylength are not directly related. Once daylength begins to decrease (June-October) mannitol continues to increase giving a pattern similar to that of mannitol v seawater temperature (Fig. 7 xxi). From July-September this is a significant negative correlation ($P < 0.02$).

With L. digitata, again there is no clear pattern during January-June, when mannitol forms a series of peaks and troughs as daylength increases. From July-September there is a similar negative correlation ($P < 0.10$) but from September-December the positive correlation may indicate mannitol content decreases in response to decreasing daylength.

Therefore, in both species, for most of the year, no definite relationship between mannitol and daylength or between mannitol and seawater temperature is apparent.

h) Mannitol Content and Seawater Nitrate Concentration

Mannitol content, in both L. saccharina and L. digitata (Fig. 7 xxiii), only begins to build up significantly when seawater $\text{NO}_3\text{-N}$ concentration is at low summer levels (May to October). Once the nitrate concentration increases in the autumn, the mannitol content declines. During the winter and spring (November-May) when seawater $\text{NO}_3\text{-N}$ concentration is high and increases to a maximum in February, mannitol content remains fairly constant and low - in L. digitata at 4.66% dry wt and in

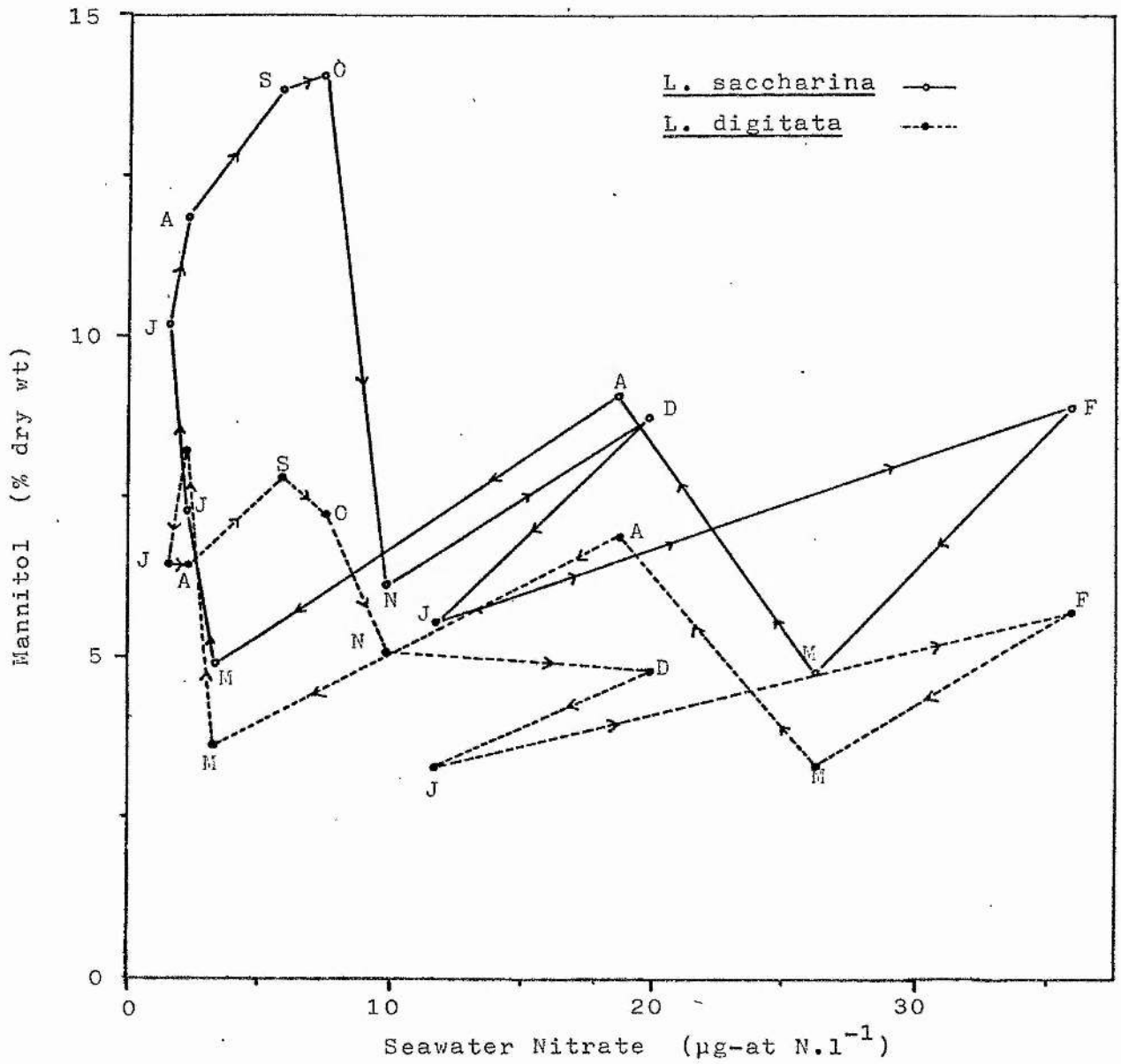


Fig. 7 xxiii. Relationship between seawater nitrate concentration and mannitol content of the meristem of L. saccharina and L. digitata at St. Andrews during 1980.

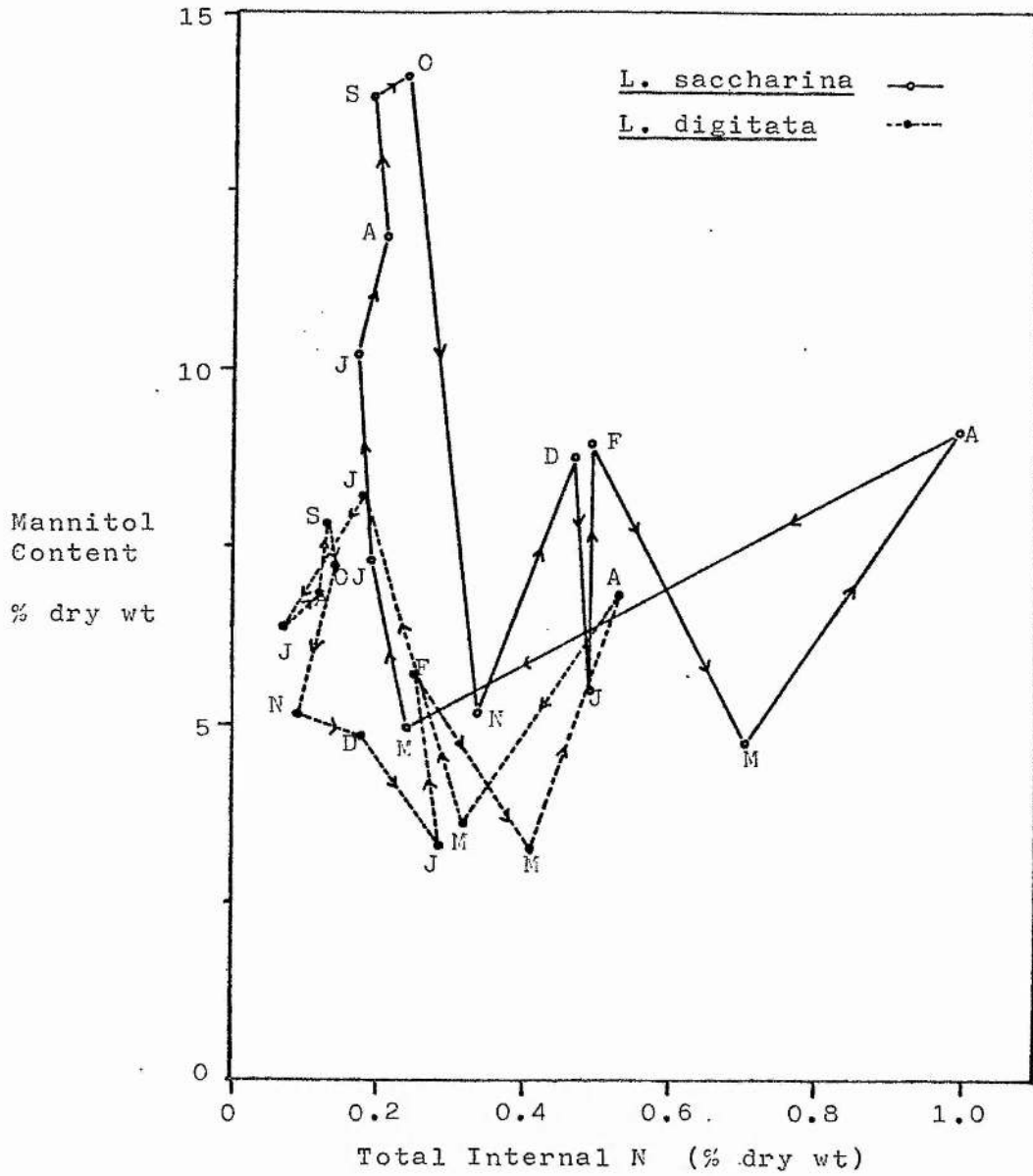


Fig. 7 xxiv. Relationship between the tissue N content of the meristem and the mannitol content of the meristem of L. saccharina and L. digitata at St. Andrews during 1980.

L. saccharina 6.88% dry wt. Mannitol content, therefore, appears to increase rapidly during nitrate depletion but a better measure of this effect would be to examine the relationship between mannitol content and internal or utilisable nitrogen reserves of the alga.

i) Mannitol Content and Internal Nitrogen (Soluble N (non-protein N) and inorganic-N) (Fig. 7 xxiv)

When internal N is at high levels, from November to April, the relationship between mannitol content and N, in L. saccharina, is unclear. However, once internal N drops to 0.243% dry wt in May, mannitol content increases very rapidly; there is a significant linear correlation ($P < 0.02$) between the 2 variables. This rapid increase continues until October, as N remains low (0.238% dry wt in October), but once internal N begins to increase mannitol drops rapidly and fluctuates about a mean value of $0.581 \pm 0.087\%$ dry wt during the winter period (November to April).

L. digitata exhibits a similar pattern with a rapid accumulation of mannitol after May, as growth rates decline, and mannitol content remains high whilst internal N is at low summer levels. After October mannitol content drops until January, and from January until May there is no definite relationship between the 2 variables.

It, therefore, appears that mannitol accumulates very rapidly as available internal N becomes limited by external nitrate concentrations. Accumulation may occur

Table 7 ii. Alginic acid content of the meristem (0-5 cm) and the more distal tissue (15-20 cm) of L. saccharina and L. digitata from the 4 sampling sites during January - March 1980. Mean \pm SE (3 replicates/treatment).

MONTH	SITE	MERISTEM % dry wt	DISTAL TISSUE % dry wt
<u>L. saccharina</u>			
January	St. A	19.52 \pm 3.71	26.36 \pm 2.81
	Sew	5.30 \pm 0.93	16.17 \pm 2.30
	KB	21.97 \pm 3.99	7.34 \pm 1.23
	FN	6.03 \pm 0.92	24.23 \pm 0.73
February	St. A	8.09 \pm 1.08	7.80 \pm 1.84
	Sew	5.29 \pm 0.66	12.42 \pm 2.61
	KB	10.83 \pm 2.69	24.86 \pm 0.25
	FN	9.71 \pm 2.31	12.68 \pm 1.54
March	St. A	13.13 \pm 0.86	-
	Sew	8.32 \pm 0.57	-
	KB	13.83 \pm 0.95	-
	FN	11.72 \pm 1.25	-
<u>L. digitata</u>			
January	St. A	7.16 \pm 0.62	12.72 \pm 0.62
	Sew	14.42 \pm 1.27	14.75 \pm 1.38
	KB	9.64 \pm 2.05	12.33 \pm 1.57
	FN	5.31 \pm 2.34	5.56 \pm 0.16
February	St. A	4.68 \pm 0.98	12.19 \pm 0.96
	Sew	8.85 \pm 1.62	14.04 \pm 2.92
	KB	5.10 \pm 0.54	18.21 \pm 5.08
	FN	8.34 \pm 1.88	8.25 \pm 0.61
March	St. A	12.94 \pm 0.74	-
	Sew	15.54 \pm 1.49	-
	KB	13.28 \pm 2.54	-
	FN	10.08 \pm 1.68	-

St. A = St. Andrews
KB = Kingsbarns

Sew = Sewer
FN = Fifeness

because growth is slowed, possibly as a result of N limitation, hence growth demand for mannitol is reduced as maximum seawater temperatures and daylengths result in maximum mannitol production.

iii ALGINIC ACID

The alginic acid content of L. saccharina and L. digitata from the 4 sampling sites, from January to March 1980 is shown in Table 7 ii. It appears that both species (and both frond tissue areas) have minimum alginic acid content in February at all 4 sites. However, there is insufficient data to draw any conclusions regarding monthly variation of alginic acid in these algae.

a) Variation in alginic acid content between the meristem and the more distal frond tissue

The results are rather variable and few trends are shown. In L. saccharina, at St. Andrews, the content of the 2 areas is not significantly different in either January or February; at Kingsbarns the meristem is higher than the older tissue in January ($P < 0.05$) but the reverse is true in February ($P < 0.05$). Whereas at Fifeness and St. Andrews Sewer the older tissue has a greater alginic acid content than the meristem in January ($P < 0.001$; $P < 0.05$) and February (NS, $P < 0.10$) at the 2 sites respectively.

In L. digitata the older tissue has a significantly higher content than the meristem in January (St. Andrews $P < 0.10$) and February (St. Andrews $P < 0.02$; Kingsbarns, $P < 0.10$)

Table 7 iii. Variation between sites in Alginic acid content in the meristem of L. saccharina.

		St. A	Sew	KB	FN
St. A	Jan		$P < 0.10$	NS	$P < 0.05$
	Feb		$P < 0.10$	NS	NS
	Mar		$P < 0.02$	NS	NS
Sew	Jan			$P < 0.05$	NS
	Feb			NS	NS
	Mar			$P < 0.10$	$P < 0.10$
KB	Jan				$P < 0.02$
	Feb				NS
	Mar				NS
FN	Jan				
	Feb				
	Mar				

Table 7 iv. Variation between sites in Alginic acid content in the meristem of L. digitata.

		St. A	Sew	KB	FN
St. A	Jan		$P < 0.01$	NS	NS
	Feb		$P < 0.10$	NS	NS
	Mar		NS	NS	NS
Sew	Jan			NS	$P < 0.05$
	Feb			$P < 0.10$	NS
	Mar			NS	$P < 0.10$
KB	Jan				NS
	Feb				NS
	Mar				NS
FN	Jan				
	Feb				
	Mar				

St. A = St. Andrews
KB = Kingsbarns

Sew = Sewer
FN = Fifeness

but there is little difference between the tissue areas at the other sites.

b) Variation between sites

L. saccharina (Table 7 iii). Plants at the St. Andrews sewer site appear to have a lower alginic acid content compared to the other sites. In January, the content is similar to Fifeness but it is significantly lower than St. Andrews and Kingsbarns. By March, the alginic acid content of these plants is significantly lower than the other 3 sites.

This lower alginic acid content in sewer plants is not seen in L. digitata (Table 7 iv), however, where these plants have a significantly higher alginate content than St. Andrews (January and February), Fifeness (January and March) and at Kingsbarns (in February).

L. saccharina from St. Andrews sewer are not only lower in alginic acid, than plants from the other sites but they are also lower in total carbohydrate (mannitol + laminarin + alginic acid. Table 7 v). This is not simply due to a lower alginic acid content but it is contributed to by a significantly reduced mannitol content at the beginning of the year. This effect may be brought about by increased carbohydrate utilisation to support the higher growth rates exhibited by these plants compared to those from the other sites. This effect is not apparent in L. digitata, however, where total carbohydrate is similar at all the sampling sites.

Table 7 v. Total carbohydrate content (mannitol + laminarin + alginic acid) as % of dry wt, in the meristem of L. digitata and L. saccharina at the 4 sites during January - March 1980.

SITE	JANUARY	FEBRUARY 1980	MARCH
<u>L. saccharina</u>			
Sewer	-	9.94	9.84
St. Andrews	25.11	17.24	17.95
Kingsbarns	27.29	16.53	19.16
Fifeness	15.22	15.24	17.80
<u>L. digitata</u>			
Sewer	-	12.43	18.42
St. Andrews	10.51	10.56	16.28
Kingsbarns	10.71	13.74	15.13
Fifeness	14.31	10.38	17.19

DISCUSSION

Alginic acid, quantitatively the most important carbohydrate, forming 73.4 and 79.5 % of the total carbohydrate in the meristem of L. saccharina and L. digitata in March, is the main component of the fibrillar cell walls of the brown algae (Hellebust & Haug, 1972b). It represents an inaccessible energy reserve, since, once formed it cannot be interconverted into other compounds

and as a result it may merely be regarded as a sideline when seasonal growth is considered. The difference in alginic acid content of plants from St. Andrews sewer compared to the other sites (L. digitata has a higher and L. saccharina a lower content) cannot easily be explained. It is not simply an effect of exposure, since St. Andrews is less exposed than Fifeness but similar in exposure to Kingsbarns. This is contrary to Haug (1964) who found alginate content of L. digitata to be higher from exposed than sheltered localities. The result for L. saccharina may, however, conform to the idea that in areas where there are adequate nutrients throughout the year, carbohydrates accumulate to a lesser degree because utilisation by growth is not curtailed to the same extent as in nutrient limited areas. Hence, these plants are lower in total carbohydrate. This aspect will be discussed further in relation to the soluble, readily utilisable carbohydrates, mannitol and laminarin.

The seasonal pattern of both mannitol and laminarin agrees with that reported by other workers for L. saccharina (Black, 1948, 1950; Black & Dewar, 1949; Haug & Jensen, 1954; Johnston et al, 1977) and for L. digitata (Black, 1948, 1950; Haug & Jensen, 1954; Jensen & Haug, 1956). Other members of the Laminariales show a similar seasonal pattern, eg L. hyperborea (see, for example, Black, 1948; Jensen & Haug, 1956),

L. longicruris (Chapman & Craigie, 1978); L. religiosa and L. japonica (Yokoyama et al, 1980); L. solidungula (Chapman & Lindley, 1980). This appears to be the general pattern in the brown algae (Craigie, 1974).

Mannitol builds up to a maximum in late summer as seawater temperature is at a maximum and the days are long but decreasing. High photosynthetic rates at this time results in maximum mannitol production but accumulation would only be expected if utilisation is curtailed. Nitrate depletion in the seawater in April causes a very rapid reduction in soluble-N in the thallus. Protein synthesis probably becomes limited by the very low tissue nitrogen content, which causes a slowing in frond growth rate in Laminaria from May onwards. It is the slowing in frond growth brought about by a reduction in protein synthesis, rather than simply a reduction in protein synthesis, which limits carbohydrate utilisation, since protein comprises only a very small percentage of the dry weight of the thallus (1.8 % dry wt in April in L. saccharina and only 0.3% in August). As growth rates are reduced the carbohydrates are no longer diluted by rapid cell division and cell enlargement, hence mannitol content increases due to this curtailment of utilisation as mannitol production is at a maximum.

A decline in mannitol content during July/August was observed in both L. saccharina and L. digitata. Black (1948) suggested such a drop represented a decline

in photosynthesis resulting from the absence of nutrients (nitrates and phosphates) in the seawater. However, he presented no experimental evidence to substantiate this and the decline was observed only in plants collected from sea lochs but not from open sea plants. Johnston et al (1977) showed such a decline in photosynthesis during May which was attributed to "poor" (unspecified) water conditions.

Evidence presented earlier (Chapter 6) showed that the photosynthetic potential of L. digitata declines after N and P become depleted in the seawater, possibly supporting the view of Black (1948) and Johnston et al (1977) that photosynthetic production is reduced, available mannitol is utilised and a drop in mannitol content is observed. However, mannitol production is controlled ultimately by the enzymes involved in the last steps of its biosynthesis (Ikawa, Watanabe & Nisizawa, 1972). Küppers & Weidner (1980) looked at one such enzyme, mannitol-1-phosphate dehydrogenase (mannitol-1-P DH) in L. hyperborea. They showed, in the meristem, that after a weakly developed spring maximum in April, the amount of enzyme increased from June and continued increasing until January - March of the next year. Not only did the total amount of enzyme increase during the summer but mannitol-1-P DH exhibited increasing activity (not capacity) during the summer to a peak in September/October. Therefore, mannitol production is maximal during the

summer and peak in activity coincides with the observed peak in mannitol content in Laminaria. Since the enzyme amount remains high despite low external summer nitrogen levels, the influence of seawater and hence, tissue N content, cannot simply be a deficiency-effect. Kuppers & Weidner (1980) attribute this effect to a specific nitrogen-triggered mechanism. Whatever the mechanism involved, it appears that the summer drop in mannitol content in L. saccharina and L. digitata cannot simply be attributed to nutrient limitation and some other factor(s) must be involved.

During most of the year there is competition for the available mannitol from 2 sources - for the synthesis of laminarin (by conversion from mannitol) and for growth (for protein synthesis, respiration etc.). Since growth and laminarin appear to be inversely related ie. laminarin accumulates as growth declines and vice versa, this competition is almost continuous. The summer drop in mannitol may be partly brought about by the diversion of available mannitol, formerly being used extensively for growth, into laminarin synthesis, as the laminarin content of the thallus of L. saccharina and L. digitata begins to increase during July and August, as growth rates decline. However, the increase in laminarin content is not sufficiently large to account for the observed decline in mannitol content. Therefore, the summer mannitol drop cannot be explained adequately

in this way.

During the summer months, mannitol content and growth rates are only indirectly related; mannitol accumulates because growth is limited by protein synthesis which is itself limited by reserve and external nitrogen. Utilisation of mannitol is curtailed at a time of maximum mannitol production, hence mannitol content increases to a peak in late summer. The subsequent decline in mannitol content occurs as mannitol production is reduced as seawater temperature and daylength limit photosynthetic production whilst increasing external and tissue N and P no longer restricts carbohydrate utilisation. Cell division and cell enlargement continue at low rates during the period of slow growth in L. digitata and L. saccharina, mannitol in the meristem is presumably channelled into maintaining this growth and into laminarin synthesis. In the more distal frond tissue the mannitol content also declines. There are 3 possible fates of the mannitol in this area where the cells are no longer actively enlarging or dividing:

- i Translocation
- ii Losses by respiration and the biosynthesis of other compounds
- iii Losses to the seawater by "secretion" or leakage

There is ample evidence that translocation does occur in L. saccharina and L. digitata. Mannitol (the

principal component of the translocate) and amino acids are transported at significant rates and in significant quantities for this to be a very important consideration (see for example, Luning, 1969; Luning, Schmitz & Willenbrink, 1972). Unlike L. hyperborea, L. saccharina and L. digitata continued growing during the second half of the year and translocation similarly continues throughout the year (Luning et al., 1973); growth and translocation must, therefore, be related. The evidence from translocation studies suggests that translocation is controlled by "sink" consumption rather than by source content, and even during the slow growing season the active meristem continues to act as a sink. Schmitz & Srivastava (1975) were able to reverse the direction of translocation in Alaria marginata by predarkening a distal portion of the frond to produce a strong enough sink to attract assimilates from normal "sink" regions, thus indicating this importance of sink consumption rather than source content in translocation. Basipetal translocation appears to be essential for growth throughout the year in L. saccharina, from evidence presented by Johnston et al. (1977). Carbon input by photosynthesis and demand from the meristematic region (basal 0-15 cm) of the frond indicated a carbon deficit throughout most of the year, implying that translocation from the mature frond tissue was necessary to balance this discrepancy.

Translocation, then seems to be a possible explanation for the observed decline in mannitol content in the mature frond tissue.

Mannitol has been shown to be a relatively minor respiratory substrate in L. digitata (Hellebust & Haug, 1972b), amino acids being more likely sources of carbon for respiration. Milthorpe (1949) found no significant change in the concentration of either mannitol or laminarin during dark starvation; however, the large quantity of mannitol present is likely to conceal respiratory losses in short-term experiments. The inter-conversion of mannitol to other compounds occurs but the extent of such biosynthesis is not known. The data did not indicate a specific level of mannitol in the tissue after which laminarin accumulates although it is possible that some minimum content is involved, and evidence presented by Black (1948) to support this hypothesis was inconclusive as laminarin accumulated at significantly different mannitol concentrations during the 2 years studied (1945-46). Hellebust & Haug (1972a) showed that the extent to which mannitol went into the biosynthesis of other compounds was very limited in L. digitata, and then less was converted in the old tissue than the younger tissue, indicating the possible senescent state of this tissue.

Losses of mannitol as a result of respiration or the biosynthesis of other compounds is, therefore,

probably of lesser importance than translocation.

A proportion of the endogenous carbohydrates are lost to the seawater by leakage of "secretion". Secretion is the term used by Johnston et al (1977) although they present no experimental evidence to demonstrate that it is active secretion rather than leakage. The extent to which carbohydrate losses occur depends on the experimental conditions tested by different workers (see Brylinsky, 1971; Moebus & Johnson, 1974; Sieburth, 1969; Sieburth & Jensen, 1969; Khailov & Burlakova, 1969). In situ studies with L. saccharina indicate high levels of carbohydrates and polyphenolic material (phluoroglucinol based) are released in late summer (Johnston et al, 1977), this amounts to over 30 % of the gross carbon fixed is "secreted" in the autumn (averaging 13 % loss over the year) with 10 % lost as leakage from the distal tissue. Leakage would be expected resulting from cell senescence and tissue damage, particularly during severe winter conditions and perhaps some of the 30 % quoted as "secreted" would be better included with the figure for leakage. Even so, the value for leakage represents a substantial loss to tissue carbohydrate content and is then likely to be an important consideration.

It, therefore, appears that translocation (which occurs at significant rates and in significant quantities during the autumn and winter in L. saccharina and L. digitata) and loss to the seawater through senescence

and damage by wave action are the 2 most important causes of the depletion of mannitol from the distal frond tissue during the autumn and winter. Losses by respiration and the synthesis of other compounds occurs but at rates which are probably insignificant relative to losses by the other 2 means.

Despite the depletion of soluble carbohydrate content during the winter in both the mature and meristematic tissue, levels even at the minimum content in February/March are always relatively high (approximately 89 mM) in both L. saccharina and L. digitata. This would indicate that soluble endogenous carbohydrates are always adequate for growth demands. This is supported by the fact that rapid spring growth begins when carbohydrates are at their lowest levels and the new frond must rely almost completely on the accumulated reserves to support growth.

However, levels of mannitol are slightly lower and laminarin are considerably lower than is recorded for L. saccharina and L. digitata found on the West coast of Scotland (Black, 1948; Black & Dewar, 1949). The laminarin content may be lower for a number of reasons:

- i) the thallus is lower in total carbohydrate, and laminarin is, therefore, formed to a lesser extent because mannitol content rarely exceeds the minimum required for laminarin synthesis.

- ii) Growth and laminarin, which are both consumers of mannitol, are inversely related. As a result of the continuous utilisation of mannitol for growth even during the period of slow growth, there is less mannitol available for the synthesis of laminarin. However, there is no indication that seasonal growth rates are any different here and that mannitol demand to support growth any greater and hence synthesis of laminarin any lower than elsewhere.
- iii) Nutrients are less limiting to growth here compared to elsewhere.

Nutrients and particularly nitrates, are important in indirectly controlling carbohydrate content through growth; in areas where nutrients are readily available, carbohydrate content would be expected to be lower because utilisation is not curtailed. This is supported by observations from St. Andrews sewer site where the higher N concentration supported rapid growth rates and carbohydrate content was correspondingly lower in L. saccharina. However, even at this site nitrogen becomes limiting during the summer and both laminarin and mannitol accumulate. This supports Black's (1954) findings of low/absent laminarin in plants from areas

with adequate nutrients throughout the year (as a result of pollution or upwelling). However, since nutrients become limiting at all 4 sampling sites during the summer this does not adequately explain the lower carbohydrate content of these plants.

Black (1948) reported that increased growth rates due to exposure result in low mannitol levels; since water turbulence reduces the width of the boundary layer around the algal frond and facilitates mineral nutrient and bicarbonate uptake, this would be expected. This effect is not shown here where St. Andrews and Kingsbarns are less exposed than Fifeness. The laminarin content is, therefore, presumably lower because the mannitol content is lower, but there is no apparent explanation for this lower mannitol content unless both nutrient levels and exposure are higher at these sites than in the other locations investigated.

In conclusion, all the evidence presented suggests that soluble carbohydrates are not limiting (either directly or indirectly) to growth of L. saccharina and L. digitata during the year. At the beginning of the year the requirements to sustain the very rapid new frond growth rates exceeds production of mannitol and tissue content declines to a minimum. Rapid cell division and cell enlargement would dilute the carbohydrate present in addition to rapid utilisation as production is minimal. Carbohydrates accumulate during the summer because growth

is limited by external, and subsequently internal N and P, restricting utilisation at a time of maximum mannitol production.

In the autumn and winter, translocation basally and loss to the seawater probably account for the decline in carbohydrate content in the mature frond tissue and in the meristem to support the continual growth as daylength and temperature decrease.

CHAPTER 8

GENERAL DISCUSSION

Laminaria is one of the most important genera in the lower intertidal and sublittoral euphotic zone, both economically (through alginate extraction) and ecologically, contributing a major proportion of the primary production in inshore waters, forming the basis of many detritus and filterfeeding food chains and providing additional substrata for attachment by sedentary organisms. It is widespread in temperate and polar waters and dominates its environment. Where Laminaria spp. occur, only members of the Lessoniaceae completely out-compete them.

A characteristic of the genus Laminaria in north temperate areas is its rapid growth in late winter and early spring before declining to a quiescent period in late summer and autumn. Phytoplankton biomass declines markedly during the same period in the spring (April/May) which is attributed directly to the nutrient decline in spring seawater (Ryther & Dunstan, 1971). This nutrient control of phytoplankton biomass is again evident in the autumn when seawater nutrients and subsequently phytoplankton biomass increase. Phytoplankton in freshwater are subject to the same nutrient control of biomass (Lund, 1950).

The seasonal growth cycle of L. saccharina and L. digitata is adapted to take full advantage of available nutrients and as with phytoplankton, nutrients may provide

an important growth controlling factor. Hence, in L. saccharina and L. digitata spring growth occurs when seawater nutrients (N and P) are abundant and growth rates fall off as nutrients are depleted to limiting summer levels (as shown here in figs. 4 xiv-xv and 5 xiii, which illustrate this correlation).

During the period of maximum external nutrient concentrations 'luxury consumption' and storage of N and P compounds occurs; nutrients begin to accumulate in the tissue shortly after external concentrations increase in the autumn with maximum tissue content in February/March for P compounds and April for N compounds. The ability for 'luxury consumption' distorts the close correlation between ambient nutrient concentrations and growth which is exhibited by the phytoplankton. This results in a time lag in the spring between the seawater N and P decline (April) and the slowing of Laminaria growth rates (May/early June). These nutrient reserves accumulated in autumn and winter are important in supporting continued growth during this lag period for up to 1½ months. Evidence for this lies in decreasing proportional N and P content with time, ie. N/dry wt and P/dry wt ratios fall.

Despite this temporal displacement the decline in frond growth rates is shown to be a direct effect of the spring N and P decline; growth rate decline was prevented by experimentally maintaining high external nutrient levels in the laboratory (Chapter 6) and in situ (Chapman & Craigie, 1977). Experiments were not carried out to

investigate the relative importance of nitrate and phosphate in this growth rate decline. Other studies indicate only nitrogen to be involved (Chapman & Craigie, 1977; Chapman & Lindley, 1980; Calvin & Ellis, 1981). At this study site, however, it is proposed that both N and P are involved for the following reasons:

- i there is a significant depletion of both N and P in the seawater in April,
- ii tissue reserves of both N and P are utilised during the lag period,
- iii there is evidence that both N and P limit summer growth of Laminaria (Chapter 6); external concentrations of both N and P are then likely to limit growth from April onwards.

There are a number of indications that concentrations of both N and P are limiting to growth of Laminaria during the summer. Enhanced frond growth rates of L. saccharina result from the addition of winter concentrations of both nitrate and phosphate in the laboratory in June/July. Separately, N and P enrichment had little effect on growth thus emphasizing the involvement of both nutrients in the low summer growth rates.

External nutrient limitation is also indicated as internal reserves continue to be utilised for growth and diluted by other cellular constituents at a rate faster than replenishment through uptake, as evidenced by decreasing relative and actual tissue N and P content. This

results in Laminaria becoming most nutrient limited (both externally and internally) by August (this is the month of minimum relative tissue N and P content in conjunction with minimum seawater nutrient concentrations). Although both N and P are taken up efficiently from low external concentrations (indicated by low $K_{1/2}$ values) further uptake may be more strongly limited by supply of the ions to the frond surface. This is particularly critical during the summer when turbulence is at a minimum and seawater nutrient concentrations are low.

The seasonal pattern of carbohydrate reserves of Laminaria also points towards summer nutrient limitation. Light (photoperiod and irradiance) and seawater temperatures are at non-limiting levels for growth during the summer (as indicated in results of Fortes & Luning, 1980) but nutrient depletion evidently limits efficient utilisation of light for this purpose. Photosynthetic production is maximal at this time (Johnston et al, 1977) and carbohydrate reserves increase significantly (Chapter 7). Such an accumulation of mannitol would occur only if utilisation is curtailed. Restriction of mannitol utilisation for growth appears to result from mineral nutrient limitation since mannitol content increases rapidly once tissue N and P content declines after depletion of seawater N and P (Fig 7 xxiii illustrates this correlation). Nitrate depletion in the seawater in April causes a very rapid reduction of soluble-N in the thallus (figs 4 vi-xi) which, by limiting protein synthesis may cause the slowing

of Laminaria frond growth rates from May onwards. Relative tissue protein content declines during this period (figs 4 vi-xi) suggesting dilution of the protein already present by other cellular constituents without further extensive protein synthesis. A reduction in protein synthesis per se is unlikely to limit carbohydrate utilisation as protein comprises only a small percentage (0.25-1.5 %) of the dry weight of the thallus, but it is proposed that it is the restriction on growth, as a result of N limitation restricting protein synthesis, which limits carbohydrate utilisation.

If this explanation for the slowing of growth rates is correct, P depletion would appear relatively unimportant in causing the spring growth rate decline. As external phosphate concentrations are depleted, internal P reserves are utilised to maintain this growth and expansion during the lag period (as indicated by a decline in the P/dry wt ratio). By May, internal reserves are largely depleted and further uptake is likely to be limited by supply of the ions to the frond surface.

P limitation may affect growth in 2 main ways:

(i) a direct effect on basic energy metabolism and (ii) an effect on membrane synthesis.

(i) Organic P in the cell may occur predominantly in ATP. Growth and expansion of the frond produces a requirement for new chloroplasts and mitochondria which, in turn produces a requirement for P; each new organelle probably requiring a minimum amount of P (for ATP, NADP etc.). It

may quickly reach the point where there are insufficient internal P resources to meet these requirements; both photosynthesis and respiration would be affected. This, in turn, would affect protein synthesis which is already seriously curtailed by nitrogen limitation (as discussed above).

(ii) P is an important component of cell membranes. As a result, P limitation might be expected to have a direct effect on cell division and cell expansion and so also, on frond growth. If cell expansion requires less P than cell division (because the membrane with its P component is already formed; expansion may simply be an expansion of this membrane rather than de novo synthesis of membrane as occurs during cell division) then P limitation might change the main form of frond growth from cell division to cell expansion. Such a proposed change is supported by evidence from Kain (1976) in which growth of L. hyperborea changes from principally cell division to cell expansion once growth rates decline after the spring maximum. A change in mode of growth is also shown here, where, at the end of the year growth is predominantly by cell expansion whilst early new frond growth is predominantly by cell division (as illustrated in Table 3 vii).

P limitation may then be important during the spring and summer in affecting both basic energy metabolism and membrane synthesis. P limitation appears to act in conjunction with N limitation during that time.

There is competition for the available mannitol from

a number of different sources; these can perhaps be arranged in the following hierarchy in an attempt to explain the seasonal pattern of carbohydrate reserves in Laminaria:-

- (i) Respiration
- (ii) Protein synthesis
- (iii) Growth
- (iv) Vacuolar storage
- (v) Laminarin synthesis
(by conversion from mannitol)

Demand for respiration is greatest in the winter; during the summer, with maximum net photosynthetic production (Johnston et al, 1977) only a small proportion of the available mannitol may be utilised in this way. External and subsequently internal limitation of N and P during the summer reduces protein synthesis (both N and P are involved in effects on protein synthesis, as discussed above) and this also reduces the requirement for mannitol. Linked to (ii), once growth rates decline, through nutrient depletion, the requirement of mannitol for growth is lessened. Consequently, respiration, protein synthesis and growth might be relatively unimportant as consumers of mannitol; available mannitol may then be channelled into vacuolar storage and laminarin synthesis. Storage clearly occurs, as evidenced by the summer accumulation of mannitol in Laminaria, reaching maximum content by September/October. Synthesis of laminarin is proposed to occur once mannitol concentration exceeds a certain threshold level (Black, 1948). Results presented here were inconclusive, but accumulation

of laminarin in late summer suggests that a threshold or minimum mannitol content might be necessary.

During the summer the accumulation of mannitol might then be interpreted as a diversion of carbohydrate reserves (through nutrient limitation of growth and protein synthesis in conjunction with adequate light for an assimilatory surplus) into storage and possible laminarin synthesis. The emphasis is presumably altered in favour of (i) and (ii) during the winter and, once new frond growth has begun in January, also into growth. There is little storage during the winter, as evidenced by the declining mannitol/dry wt. ratio and little laminarin synthesis; laminarin content declining rapidly once growth of the new frond begins.

To summarise: Seawater nutrient depletion of both N and P causes the spring decline in linear growth rates of L. saccharina and L. digitata from May onwards. No other nutrients appear to be involved since the growth decline was prevented by enrichment with N and P only. N and P continue to be limiting growth during the summer, as evidenced by:

- (i) depletion of internal reserves of N and P
- (ii) enhanced growth in June/July on N+P enrichment
- (iii) uptake of N and P being limited by supply of ions to the frond surface
- (iv) accumulation of mannitol in response to limiting tissue N and P.

Arising from the premise of nutrient limitation of summer growth of Laminaria, it might be expected that growth rates would increase in the autumn (September) as seawater nutrient concentrations increase. In support of this, growth rates of both L. saccharina and L. digitata increase, as illustrated in figs 3 i-iv, but the effect is only transitory and growth declines again by mid-October. The growth rates are less than predicted from growth rate increases in the spring at comparable levels of external N and P. Some factor(s), other than seawater nutrients, must then be responsible for the reduced or "not-increased" growth and it is proposed that senescence may be important in this context. Senescence appears to be a normal feature of Laminarian morphogenesis in cells distal to the meristematic zone and therefore, it may increase with time as summer continues into autumn.

A loss of growth potential is first apparent in mid-summer; growth of L. saccharina enriched with both nitrate and phosphate increased above the low N and P controls in June/July (Table 6 ii) but the actual growth rates expressed were less than predicted from the external N and P concentration and the corresponding tissue N and P content, as calculated from spring growth responses. By September, L. digitata showed only a very limited response to increased external N and P concentrations in the laboratory (Expt. 6 iii a) and similarly, in situ, the growth increase was small and short-lived. As is consistent with senescence,

uptake capacity is retained and accumulation of N and P from the medium occurs (in enrichment experiments) and in situ tissue N and P also increases significantly from September onwards.

Since the loss of growth potential occurs soon after the spring decline in Laminaria growth rates it is suggested that N and P depletion in the seawater or some other factor declining in parallel causes senescence of the frond tissue. As the mature frond tissue does not grow it is only the meristem which determines frond growth; nutrient depletion and loss of growth potential then must most affect this area of the frond.

On displacement of cells through growth beyond the basal 10 cm (transition zone) of the frond there is a loss of meristematic activity. This is a normal feature of Laminarian morphogenesis noted by many workers (eg. Parke, 1948; Sundene, 1964; Kain, 1976). Senescence with time through the year may simply be an extension of this tissue maturation process. Once growth rates have slowed down as seawater nutrients are in short supply, the area of the active meristem will decrease through the summer as displacement of tissue away from the meristem is reduced (by the lower rates of growth). Tissue within the basal 10 cm of the frond in, for example, September, is older than tissue in the basal 10 cm in, for example, April when rapid growth results in a rapid displacement of tissue. Perhaps by mid-late September this basally spreading wave of tissue maturation has spread through almost the entire meristematic

zone. Such a basal movement of meristematic activity has been shown by Sundene (1964) and Cosson (1967) in L. digitata where, in the spring, growth occurs between 40-100 mm from the transition of stipe and frond whilst later in the year most growth occurs in the basal 50 mm. The reduced response to increased nutrients in the late summer may simply reflect the significantly reduced meristematic area available to respond to the improved conditions. This simple explanation for the loss of frond growth potential (or senescence) is not completely adequate since even the reduced area might be expected to expand rapidly by cell division. This apparently occurs in January when, by the same argument, the meristematic area would be still further reduced than in September. In addition, this explanation cannot account for the changing response of Laminaria to light later in the year (between November and December). However, this wave of tissue maturation and reduction in meristematic area may be an important consequence of nutrient depletion which operates in conjunction with other endogenous and exogenous controls.

A programmed senescence to do with age or photoperiod, or a dormancy effect might be involved in conjunction with the initial nutrient depletion effects on growth rate. The role of photoperiod has not been studied here but it might be important since senescence is shown here to be an effect from mid-summer onwards (as indicated in the June/July enrichment experiments) and rejuvenation of meristematic activity in January is associated with increased

daylengths (as illustrated in experiments 3 vi a+b). This may suggest a simple photoperiodic control of senescence which begins as daylengths decrease from mid-summer onwards and continues until the shortest day is reached; increasing photoperiod then acts as the 'trigger' for the following year's growth to begin. This aspect of the control of Laminaria seasonal growth has received surprisingly little attention and further investigation is required on the proposed photoperiod control of new frond growth.

Little work has been undertaken on the possibility of dormancy in Laminaria although there is some evidence for the existence of growth-controlling substances in this genus. Gibberellin activity has been reported in L. digitata, L. saccharina and L. hyperborea, being at a peak in the summer (Wildgoose, Blunden & Jewers, 1978); the explanation of this at a time of cessation of fast growth is not clear. There is some evidence for the presence of kinetin-like substances in Laminaria (Hussain & Boney, 1969) and both the lamina and stipe of L. digitata contained water-soluble growth-inhibiting substances (Hussain & Boney, 1973). The relevance of these growth-inhibiting substances to the seasonal pattern of growth of Laminaria cannot be established without further information on seasonally varying levels of these substances. Certainly this field is one in which much more important work has still to be done.

Nutrient depletion alone cannot explain the loss of growth potential or senescence of L. saccharina and L. digitata; as described above it is probably important in conjunction

with other endogenous and exogenous controls. However, nutrient depletion does result in another more immediate senescence effect; that of a decline in photosynthetic capacity or potential of Laminaria. Such a decline in L. digitata might occur because enzyme levels would be expected to be significantly reduced by tissue expansion once N and P reserves have been rapidly utilised on depletion of seawater N and P in April; the minimum enzyme quantity (August - Küppers & Weidner, 1980) occurs when nutrient limitation is at its most critical. With N and P enrichment, this decrease in enzyme quantity is presumably prevented, hence photosynthetic capacity is maintained (refer to Table 6 i). As suggested from the close correlation in the spring between photosynthetic capacity and seawater nutrient concentration, there was a slow increase in photosynthetic capacity in the autumn (with increasing external nutrient levels) and high photosynthetic capacity was recovered by January in the old frond tissue before it was replaced by new frond tissue (in L. saccharina and L. digitata; Drew, pers comm.). A similar loss of photosynthetic capacity during the summer has been shown for L. hyperborea (Küppers & Weidner, 1980).

The importance of this decline is not clear since Küppers & Weidner (1980) found that highest metabolic activity of the enzymes Ribulose-1, 5-bis-phosphate carboxylase and Glyceraldehyde-3-phosphate dehydrogenase occurred in August despite a minimal enzyme quantity at this time. Maximum enzyme amounts early in the year (other

enzymes, including malate dehydrogenase and phosphoenolpyruvate, also show a spring maximum (Küppers & Weidner, 1980)) might be advantageous since it would enable Laminaria to establish relatively high metabolic rates at a time when temperature is probably limiting growth. This is assuming that there are adequate endogenous reserves from January-April and sufficient light from April onwards (after reserves are largely depleted) to support growth at this time.

Because of increased enzyme activity in summer deleterious effects of the decline in photosynthetic capacity (ie. a decline in enzyme amount) may become apparent only in the autumn as photoperiod, irradiance and seawater temperatures decrease. In L. saccharina and L. digitata where photosynthetic capacity appears to show a recovery in the autumn this decline in photosynthetic capacity seems relatively unimportant. It may represent an adaptation which conserves tissue nutrient reserves since it would be an advantage for Laminaria, once spring growth is approaching its maximum, not to have to use valuable internal N reserves for excessive enzyme syntheses to maintain enzyme quantities at high, spring levels when seawater nutrient depletion limits enzyme function.

Whatever the control mechanism involved in frond maturation, whether nutrient depletion, dormancy, senescence through age or photoperiod, or some other factor, photoperiod is clearly important at the end of the year in the

initiation of the following year's new frond growth. Increasing photoperiod after the shortest day in December had the effect of "switching on" cell division, whereas growth previous to this was predominantly by cell enlargement (results presented in Table 3 vii indicate this change from growth by cell enlargement to growth by cell division). The importance of this effect was demonstrated experimentally as new frond growth was initiated in early December (before the shortest day) by lengthening the photoperiod. This photoperiodic effect is not apparent in November where increasing the daylength resulted in growth which was not significantly greater than growth in the short day controls. The 'lack' of response to increased daylength in November adds further support to the possibility of a circannual rhythm of frond growth or of senescence of the frond tissue. Tissue senescence may explain why a new and distinct frond is produced in January each year; the existing frond is unable to respond by renewing meristematic activity in the mature tissue and only the very reduced meristematic zone can respond to the increasing daylength.

Increasing photoperiod may act as the "triggering mechanism" for cell division to be "switched on" and for growth rates to increase yet the newly initiated growth is partially limited at the time by total irradiance and seawater temperature, and ambient photoperiod is probably inadequate for the production of sufficient organic materials (this is the case in L. hyperborea; Luning, 1971). As a result, the alga must rely on stored reserves as well to

meet the total new frond growth requirements. Despite the increased mechanical stresses to which the stipe and holdfast are subjected during the winter, the strategic advantage of retention of the previous year's senescent frond is clear in providing reserves to support new frond growth. The role of summer-accumulated carbohydrate reserves in supporting new frond growth is well documented (Luning, 1969) and there is ample evidence to show that translocation of mannitol occurs from the distal to the young tissue at this time (see review; Schmitz & Lobban, 1976). Utilisation of carbohydrates is indicated indirectly in L. saccharina and L. digitata here by a decrease in dry weight/unit area of frond and by a decreased carbohydrate content (particularly mannitol and to a lesser extent laminarin) of both the mature and the new frond tissue. The actual contribution of the translocated materials to new frond growth is under controversy (see review; Kain, 1979) but most of the evidence suggests that retention of the old frond (or a large proportion of it) is important for normal spring growth of L. saccharina and L. digitata.

The old frond is shown to have several supportive functions in addition to acting as a storage organ for carbohydrates accumulated in the previous summer. The old frond provides an increased area for nitrate and phosphate uptake, basal translocation then brings these nutrients to the actively growing new frond. This role may be particularly important since there is a suggestion that ion uptake capacity (as indicated in section 3 viii c) and

possibly also the velocity of uptake by the growing tissue are very much less than that of the mature non-growing tissue. This would imply that the new frond may be dependent, to a large extent on mineral supply from the old frond, at least until a sufficient volume of new frond tissue had been displaced beyond the basal meristem. In the absence of the old frond, either experimentally or by natural attrition, and despite high external nutrient concentrations, the new frond may be severely nutrient limited. In the short-term experiments investigating growth of new fronds after excision of the old frond, internal reserves of N and P declined in ambient seawater treatments (Tables 3 xii+xv) suggesting current uptake was insufficient to support growth demands in January. Over a longer period internal nutrient limitation might reach the extent of slowing new frond growth rates, despite maximum external N and P concentrations. In conjunction with increased area for nutrient uptake, the old frond provides a pool of N and P compounds accumulated in the tissue from September onwards as seawater N and P concentrations increase. Retention of much of this nutrient-rich tissue (despite distal tissue erosion more than 50% of the frond tissue present in October remains attached by the following January see figs 3 xvi + xvii) accompanied by basipetal translocation (demonstrated by Schmitz et al, 1972; Floc'h & Penot, 1971) provides a substantial reserve available to the developing new frond.

A fourth possible role of the old frond is in increasing

the area for photosynthesis. Although for long periods during the winter irradiance is probably below the light compensation point for photosynthesis (information on the actual amount of light received by these lower intertidal algae is lacking) an increased photosynthetic area would be advantageous to the new frond when irradiance is sufficient for a photosynthetic surplus. The relationship between growth and photosynthetic area may not be a simple function of total photosynthetic area (as in L. longicruris; Chapman & Craigie, 1978) and although growth rate is a linear function of frond length (up to 50 cm frond length) in L. saccharina (Luning et al, 1973) the basal meristem is partly dependent on its own assimilation. In one case the basal 12% of the frond length assimilates 30% of the carbon required for growth, 70% is translocated from the mature frond tissue (Luning et al, 1973). It is, therefore, difficult to distinguish the effects of translocation from those of photosynthetic production of the old frond.

The old frond is, therefore, apparently necessary for normal new frond growth in late winter and spring in acting as a storage organ for carbohydrates and both N and P compounds and increasing the surface area for nutrient uptake and photosynthesis. In addition it may supply hormones and growth-promoting substances to the new frond which act in combination with the other functions described above.

To summarise; nutrients are important in controlling the seasonal growth pattern of L. saccharina and L. digitata

particularly during the first half of the year when the decline in Laminaria growth rates is a direct effect of seawater nutrient depletion. The role of light is, as described above, not completely clear. Irradiance and photoperiod are likely to limiting growth during the winter months but increasing photoperiod after the shortest day appears to act as the trigger for initiation of new frond growth in January. It is possible that photoperiod is involved in frond senescence during the latter half of year. Temperature limitation of growth appears to be effective during November-April but since growth rates increase from January onwards the effect of low temperatures may only be important during November and December.

As a result of the importance of nutrients in controlling the growth pattern of Laminaria both the actual growth rates expressed and the seasonal pattern of growth (particularly with regard to growth during the summer months and to the timing and extent of new frond growth) might be expected to vary between locations which differ in seawater nutrient conditions. Although it was anticipated that the increased sewage discharge during the summer might maintain high N and P concentrations throughout the year, this was not found to be the case. In consequence, the seasonal growth pattern of L. saccharina and L. digitata did not differ markedly from that at the 3 lower nutrient sites (at St. Andrews, Kingsbarns and Fifeness), although growth rates of both L. saccharina and L. digitata were significantly higher during the spring at the Sewer site.

Evidence presented earlier indicated summer nutrient limitation of Laminaria growth; in areas of 'adequate' summer nutrient levels the growth rate decline might be expected to be prevented and Laminaria might maintain high growth rates throughout the summer. 'Adequate' nutrient concentrations being levels which result in no large fluctuations in tissue N and P content; the N/dry weight and P/dry weight ratios might be expected to remain at levels found here (see figs. 4 vi-xi and 5 v-xii) during the winter. It was not possible to show this in the field either at the Sewer site or by in situ fertilisation experiments (no such experiments were successfully completed). Chapman & Craigie (1977) were able to prevent the summer decline of L. longicruris growth by in situ nitrate fertilisation and recently it was demonstrated that high and continuing mineral nutrient availability enabled L. longicruris to take advantage of summer light and temperature conditions to grow rapidly through the summer at a location (Bic Island, Quebec) which has elevated seawater nutrients, especially N, all year (Anderson et al, 1981). In agreement with increased rates of growth at nutrient enriched sites (eg. St. Andrews Sewer) the maximum growth rate of L. longicruris from Bic Island (Anderson et al, 1981) was 4 times that of L. longicruris from a summer nutrient-depleted location (Nova Scotia; Chapman & Craigie, 1977).

Varying the external nutrient concentration clearly has an important effect on the seasonal growth pattern of

Laminaria and high nutrient levels can apparently prevent the spring growth rate decline and allow rapid growth during summer light and temperatures. However, as discussed above, without the summer curtailment of growth there is probably little accumulation of carbohydrate reserves, which might then be expected to affect subsequent new frond growth (as indicated by the removal of carbohydrate reserves by excision; figs 3 xiv + xv). Certainly carbohydrate levels are reduced in areas of higher nutrient concentrations throughout the year (see St. Andrews Sewer results - figs 7 iii, iv, xi, xii, and Black, 1954). Anderson et al (1981) showed little carbohydrate accumulation in L. longicruris in such areas and Chapman & Craigie (1977) similarly detected little accumulation by L. longicruris during summer N-enrichment. The effect of this on subsequent new frond growth is not clear since the summer nutrient decline at the Sewer site resulted in a significant accumulation of mannitol and Chapman & Craigie (1977) did not extend their enrichment experiment sufficiently for the effects of depleted carbohydrate reserves on the subsequent period of rapid growth, to be indicated. However, results from Anderson et al (1981) indicate that rapid growth begins only after the ice cover (presumably there was also snow covering the ice to reduce light penetration) has disappeared in February. This may suggest that the new growth, unable to depend on internal carbohydrate reserves, can only begin growth when there is sufficient light for photosynthetic production to meet growth demands.

A similar temporal displacement of new frond growth (this is a significant time lag in L. longicruris, where rapid growth begins in November in summer N-depleted locations; Chapman & Craigie, 1977) might occur in L. saccharina and L. digitata in locations with maintained high nutrient concentrations, although further information is also required on winter in situ irradiances before this aspect of seasonal growth can be resolved.

The results from St. Andrews Sewer highlight some of the problems of interpretation of factors controlling growth rate and the absolute rates of growth cannot necessarily be compared. The populations may differ in age and the position and height of plants on the shore, exposure of the site, currents and upwelling are all important in addition to the concentration of seawater nutrients. Laminaria at the Sewer site are at extreme low water of spring tides (ELWS), during parts of the winter (December to February/March) they are only emersed for short periods during each tidal cycle or sometimes not at all. As a result there is an almost continuous movement of water passed the frond surface (except when emersed) which effectively reduces the width of the boundary layer (Wheeler, 1980) and enhances nutrient (N, P and bicarbonate) uptake (Whitford & Schumacher, 1961; Doty, 1971; Neushal, 1972). Nutrient uptake of Laminaria higher up the shore (at St. Andrews and at Fifeness and Kingsbarns) during periods of emersion is restricted to the diffusion of ions retained in the film of moisture on the frond surface. At the Sewer

site there are both enhanced nutrient concentrations and increased nutrient availability to the algae.

In a similar way the degree of exposure of the site will determine water movement around the frond and vary the nutrient supply for uptake. During the summer when nutrient uptake may be limited by supply of the ions to the frond surface it would be advantageous for Laminaria to grow in the more exposed sites. However, this must be balanced with the danger of the frond or the whole plant being dislodged during storms and bad weather in the winter and the probable increased frond erosion at exposed sites, resulting in a reduction in accumulated reserves available for new frond growth. In this instance neither growth rates (Figs 3 ii + iv) nor tissue N or P content (Figs 4 xb + xib and 5 vi + x) of Laminaria were higher from the exposed site (Fifeness) than from the more sheltered locations (Kingsbarns and St. Andrews). Increased exposure, therefore, may not compensate for lower nutrient levels.

Competition between Laminaria spp. and other macroalgae for available nutrients must occur as evidenced by the similarity in seasonal variation of tissue nitrate (eg. Alaria esculenta, Fucus vesiculosus, F. serratus; Larsen & Jensen, 1957) and tissue protein content (Fucus spp, Black, 1949); Ascophyllum nodosum (Black, 1948) Dictyota dichotoma and Sargassum vulgare (Munda, 1962)). Although fewer species have been investigated in relation to tissue P content, competition probably also exists as Fucus virsoides

(Zavodnik, 1973) exhibits a similar seasonal pattern to L. saccharina and L. digitata shown here. L. saccharina and L. digitata have the advantage over the Fucaceae and other macroalgae in beginning growth earlier in the year, by dependence on accumulated carbohydrates (which overcomes light and temperature limitation effects), thereby utilising nutrients directly for growth whilst these are at maximum seawater concentrations. This differs from the seasonal growth pattern of many other macroalgae in which maximum growth occurs from March through to the end of the summer.

Assuming that there are the same summer nutrient limitation effects on these algae, a much greater proportion of the rapid growth period must rely on stored nutrient reserves; possibly only during March to mid-April are external nutrient concentrations sufficiently high to support growth directly. External nutrient concentrations have been shown to be more than adequate to meet Laminaria growth requirements (indicated by 'luxury consumption') from January to mid-April. Such a growth pattern means that by mid-April the Laminaria frond is sufficiently large to provide a large reserve pool of N and P compounds (accumulated throughout the winter months) and to provide an extensive surface area for nutrient uptake - an essential requirement when the diluteness of external nutrients means that nutrient uptake is limited by supply to the frond surface. Part of the ecological success of Laminaria must be in the growth pattern allowing for a large surface area

(which facilitates nutrient uptake during summer-depleted conditions) to be attained before the spring seawater nutrient decline.

In discussing competition for available nutrients between Laminaria species and other macroalgae, comparison of the ability to take up nutrients from low summer seawater concentrations is important. The ability of Laminaria to take up several forms of N simultaneously (nitrate, nitrite and ammonium as illustrated in Table 4 vii) and to take up these and phosphate in the dark are all important considerations as well as relatively low $K_{1/2}$ values for nitrate and phosphate uptake which indicate efficient uptake from low external concentrations. Although $K_{1/2}$ values have been determined for a number of macroalgae (eg. L. longicruris, Harlin & Craigie, 1976; Macrocystis pyrifera, Haines & Wheeler, 1977; Fucus spiralis, Topinka, 1978) it is likely that constants will vary with temperature (as in Codium fragile; Hanisak & Harlin, 1978) and with other environmental factors, thus making direct comparisons between the different studies difficult. Before the ecological significance of the Michaelis-Menten uptake constants of macroalgae can be fully understood uptake of many more algae should be measured over a wide range of environmental conditions. Until this has been done one can only speculate on the outcome of such competition between Laminaria and other lower intertidal macroalgae during the spring and summer.

Experimental evidence presented and the similarity between the seasonal fluctuations in phytoplankton biomass and seasonal growth of Laminaria (particularly during the first half of the year) suggests that Laminaria is in direct competition with phytoplankton for available N and P. Such competition for nutrients between phytoplankton and seaweeds was first suggested by Marshall & Orr (1949). Although there are many factors involved in algal competition other than N and P (eg. light), Laminaria and other macroalgae appear to have some advantages over phytoplankton in the competition for N and P. These include; the simultaneous uptake of several forms of N, 'luxury consumption' of N and P during the winter (which provides an important reserve when external supplies are limiting) and an increased supply of nutrients to an attached seaweed as opposed to a floating phytoplankter (demonstrated by Munk & Riley, 1952). Since nutrients are considered to be the limiting factor for growth of phytoplankton in coastal waters (Ryther & Dunstan, 1971) and evidence from this study suggests that summer growth of Laminaria may be limited by available nutrients, this competition for nutrients between phytoplankton and Laminaria (and other attached macroalgae) may be extremely important in these areas.

However, nutrients are unlikely to be the only constraints on Laminaria growth during the summer. Light (irradiance and photoperiod) and seawater temperatures

have been shown here to be at levels which are non-limiting to growth during the summer months, yet light may be indirectly affecting Laminaria growth. Space is unlimited (within the euphotic zone) for phytoplankton but this is not true of attached macroalgae where, if leaf area index (LAI) approaches the levels where light and CO₂ supply are all intercepted by the macroalgal community, competition would then become increasingly controlling of community growth rate and biomass. Although the accumulation of mannitol during the summer provides evidence against this occurring, the drop in mannitol content in the frond in June/July may indicate that the light and CO₂ supply reaching the algal frond is not always adequate.

But despite this consideration, nutrients are clearly important in controlling the seasonal growth pattern of Laminaria saccharina and L. digitata and are likely to be an important aspect of competition of Laminaria with both other macroalgae and phytoplankton. Senescence is one aspect in the control of Laminaria seasonal growth which has been seriously neglected. Evidence presented here suggests that senescence may be a major factor, particularly after the initial nutrient-depletion effects and it is an area of research which requires further investigation.

CONCLUSIONS

- 1 The decline in frond growth rates of L. saccharina and L. digitata in May is a direct effect of the decline in seawater N and P in April. No other nutrients (eg trace micronutrients) are involved. Internal N and P reserves support maximum growth rates for up to 1½ months after the April decline in seawater N and P concentrations.
- 2 Summer seawater concentrations of N and P are limiting to growth of Laminaria, evidenced by:-
 - (a) enhanced growth on enrichment with N and P during the summer
 - (b) relative depletion on internal N and P reserves
 - (c) accumulation of mannitol and subsequently laminarin in response to saturating irradiance and limiting N
 - (d) uptake of N and P being limited by supply to the frond surface.
- 3 The frond appears to undergo senescence both with distance from the meristem and with season. Senescence or a loss of growth potential is apparent in June/July and continues through until November/December. Whether senescence is endogenously or exogenously induced is not clear but nutrient limitation and photoperiod are implicated.

- 4 New frond growth appears to be initiated in January in response to increasing photoperiod. Irradiance and temperature are likely to be limiting to growth at this time and prior to new frond growth (November and December). Increasing daylength appears to 'switch on' cell division where growth previous to this was predominantly by cell enlargement.
- 5 New frond growth once started relies on reserves (N- and P-compounds and carbohydrates) accumulated in the old frond tissue during the previous months. The old frond is probably important in increasing the surface area for photosynthesis when there is sufficient light for an assimilatory surplus. The old frond also has a role of increasing the surface area for nutrient uptake, particularly important during the early stages of frond growth as the new frond may have a reduced ability for uptake of N and possibly also P.
- 6 Despite continual attrition a large proportion of the old frond (with its accumulated reserves) present in the autumn (October) remains attached and available to support new frond growth during January-March.
- 7 'Luxury consumption' of N and P occurs during the winter months. These stored reserves are utilised to support growth during the lag period between the decline in seawater nutrient concentrations in April and the decline in Laminaria growth rates in May.

- 8 Tissue N and P content follows changes in seawater N and P concentration but the actual tissue content is not simply determined by external concentration; the length of periods of emersion, the exposure of the site and water movement passed the frond surface are all important in this context.
- 9 Nitrate and phosphate can be taken up efficiently from low external concentrations (as indicated by low $K_{1/2}$ values) and can be taken up in the dark, although the rate of nitrate uptake is depressed in darkness.
- Laminaria can take up several forms of N simultaneously (nitrate, nitrite and ammonium) and at the concentration tested (winter seawater N concentrations) the rate of uptake was determined by the supply concentration.

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APPENDIX 1

PHOTOSYNTHETIC MEASUREMENTS

Reagents:40% MnSO_4

50% KI + 50% KOH

50% H_2SO_4

0.05N sodium thiosulphate

1% soluble starch solution

Calculation of Net Photosynthetic Rate

Calculations from the titre of sodium thiosulphate used (T in equation) are based on the equivalence of 1 ml 0.05N thiosulphate to 280 μl of oxygen.

μl oxygen originally in bottle

$$= \frac{280 \times T \times V}{V - 1.5}$$

where T = titre of sodium thiosulphate

V = exact volume of bottle

Sample calculation

Tissue incubated in bottles for 2 hours.

Bottle A - incubated in the light V=29.0 ml T=0.780 ml

Bottle B - incubated in the dark V=29.9 ml T=0.470 ml

Using the above equation, μlO_2 in the bottle is calculated.

Appendix 1 (continued)

Bottle	μlo_2	Change in μlo_2	Dry wt. of tissue (g)	μlo_2 g dry wt ⁻¹ .h ⁻¹
Blank*	159.40			
A	230.31	+70.91	0.0468	+757.59
B	138.55	-20.85	0.0489	-213.19

* Blank - incubated without tissue

Net Photosynthetic Rate

$$= 757.59 - 213.19$$

$$= \underline{544.40 \mu\text{lo}_2 \cdot \text{g dry wt}^{-1} \cdot \text{h}^{-1}}$$

APPENDIX 2

WATER ANALYSIS - NITRITE

Reagents:

- Sulphanilamide solution
(5 g sulphanilamide dissolved in a mixture of 50 ml HCl and about 300 ml distilled H₂O. Dilute to 500 ml with distilled water)
- N-(1-Naphthyl)-Ethylenediamine Dihydrochloride solution
(0.50 g of the dihydrochloride diluted in 500 ml distilled water)

Method

To a 50 ml water sample add 1.0 ml sulphanilamide solution, shake and allow to react for a period of 2-8 minutes. Add 1.0 ml of the naphthyl-ethylenediamine solution and mix. Measure the optical density between 10 mins and 2 hours later in 10 cm glass cuvettes on a Corning Absorptiometer (Filter 604). Distilled water blanks and standard nitrite solutions were analysed in a similar way.

Sample calculation

Seawater sample OD = 18.13

Distilled H₂O blank OD = 9.80

Corrected sample OD = 8.33

From the standard curve (Fig. A1) the nitrite conc. at OD 8.33 is 0.156 µg-at N/l.

Fig. A i. Calibration curve for nitrite in seawater.

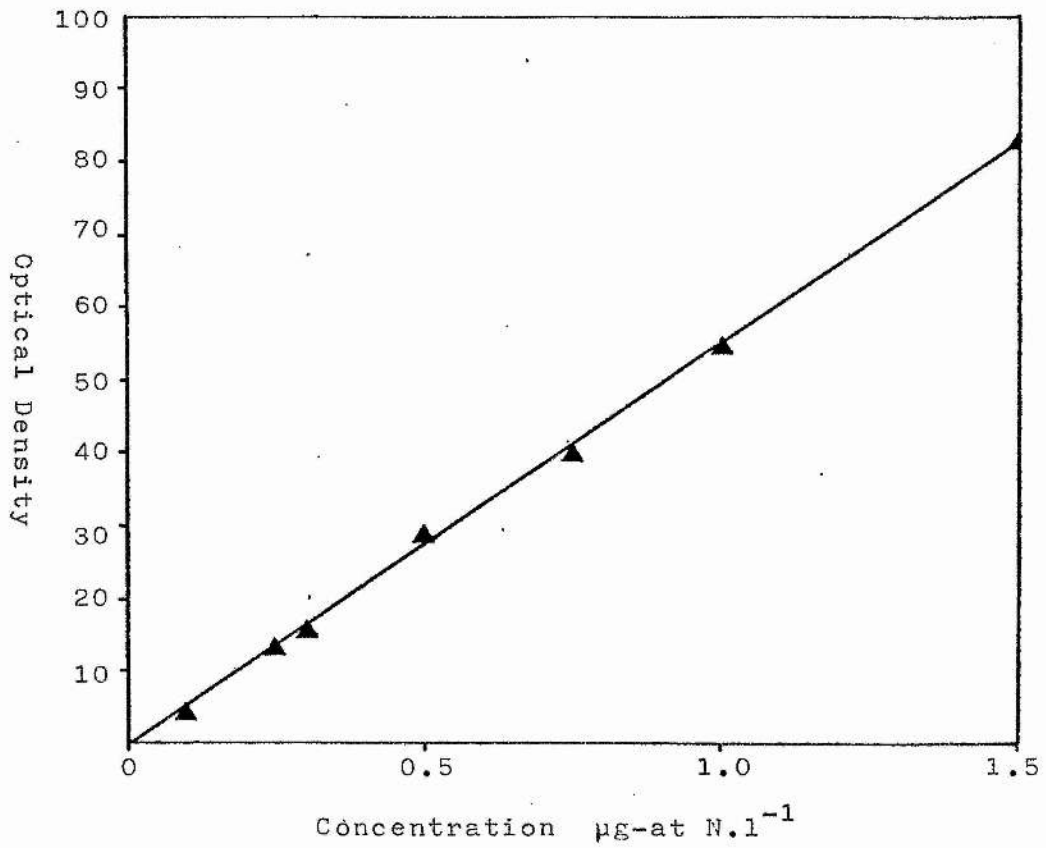
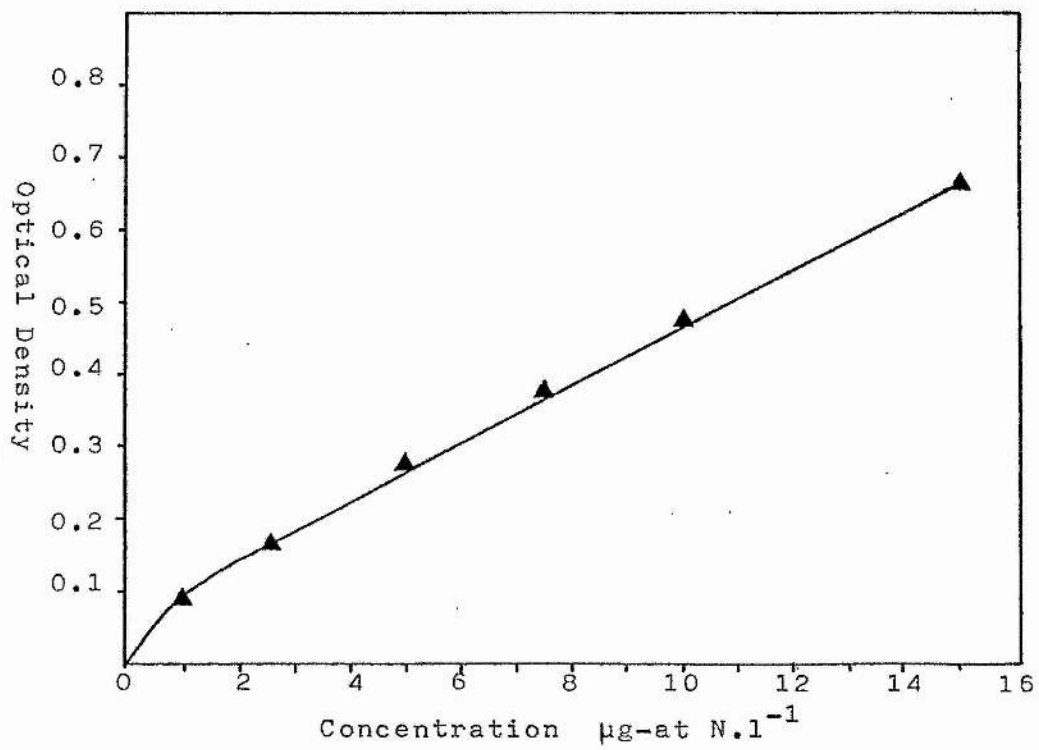


Fig. A ii. Calibration curve for nitrate in seawater.



APPENDIX 3

WATER ANALYSIS - NITRATE

Reagents:

- Concentrated ammonium chloride solution (100 g ammonium chloride in 500 ml distilled H_2O)
- Dilute ammonium chloride solution (50 ml of conc. ammonium chloride solution in 1950 ml distilled H_2O)
- Amalgamated Cadmium filings
- Sulphanilamide solution (Appendix 2)
- N-(1-Naphthyl)-Ethylenediamine Dihydrochloride solution (Appendix 2)

Method

2 ml conc. ammonium chloride solution is added to a 90 ml water sample, mixed and the sample is poured on to the column containing the amalgamated cadmium filings; the effluent is collected under the siphon. The first 25-30 ml of effluent from the column is discarded, the remainder of the sample is collected in a flask. When the flow from the sample has ceased, 50 ml of this sample is measured out. As soon as possible after reduction 1.0 ml of sulphanilamide solution is added. After 2-8 minutes 1.0 ml naphthyl-ethylenediamine solution is added and the solution mixed. The optical density is measured between 10 minutes and 2 hours later in 1 cm glass cuvettes at 543 nm. The columns were rinsed

Appendix 3 (continued)

with 25 ml dilute ammonium chloride solution between samples.

Distilled water blanks and standard nitrate solutions were analysed in a similar way.

Calibration curve for nitrate in seawater (Fig. Aii).

Sample calculation

Water sample (less reagent blank)

$$\begin{aligned} \text{OD}_{543} &= 0.250 \\ &= 4.32 \text{ } \mu\text{g-at N/l} \end{aligned}$$

But this figure also includes the original nitrite present in the water sample.

Nitrite concentration = 0.156 $\mu\text{g-at N/l}$

The nitrate concentration of the seawater sample is

$$\begin{aligned} 4.32 - 0.156 \text{ } \mu\text{g-at N/l} \\ = \underline{4.164 \text{ } \mu\text{g-at N/l}} \end{aligned}$$

APPENDIX 4

WATER ANALYSIS - AMMONIA

Reagents:

- Sodium hydroxide solution (330 g sodium hydroxide in 2000ml distilled H_2O)
- Sodium hypochlorite solution
- Sodium Arsenite solution (dissolve 20 g arsenic trioxide in about 100 ml water and 30 g NaOH. Cool the solution and dilute to 500 ml with distilled H_2O)
- Potassium bromide solution (1.5 g potassium bromide in 250 ml distilled H_2O)
- Oxidising reagent (0.75 ml sodium hypochlorite solution to 100 ml sodium hydroxide solution)
- Acidifying solution
- N-(1-Naphthyl)-ethylenediamine dihydrochloride solution (Appendix 2)

Method

10 ml oxidising reagent is added to a 50 ml seawater sample, mixed and allowed to react for 4 hours at room temperature (the tops of the flasks are covered with aluminium foil to prevent contamination by atmospheric ammonia). 2 ml sodium arsenite solution was added, the solution mixed, followed by the addition of 10 ml acidifying solution. After a period of between 3-8 mins 1.0 ml naphthyl-ethylenediamine solution is added.

Fig. A iii. Calibration curve for ammonium in seawater.

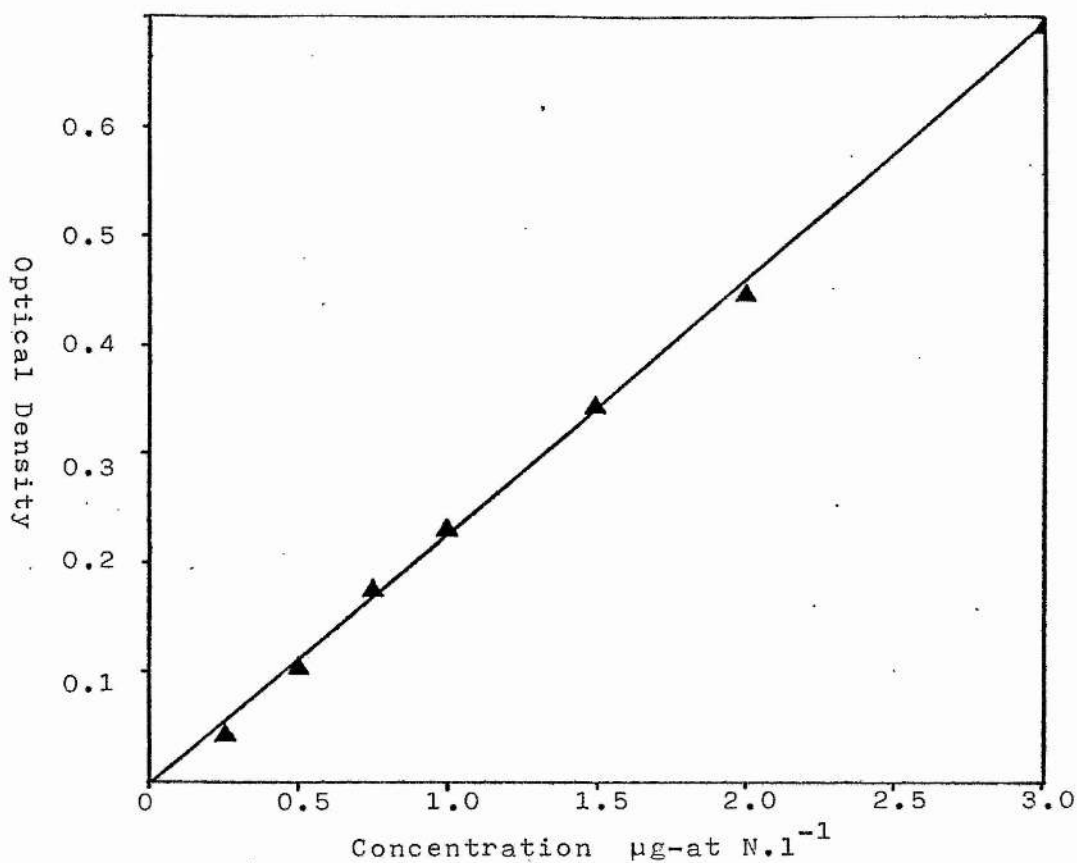
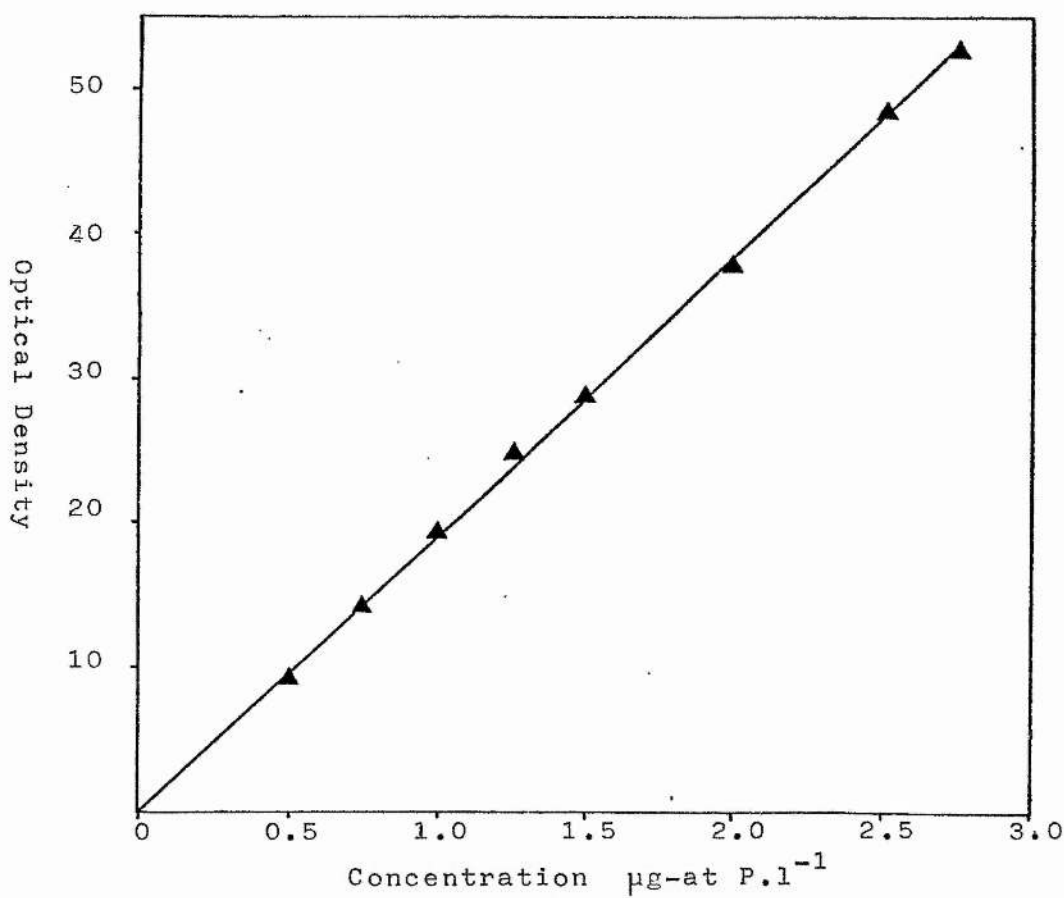


Fig. A iv. Calibration curve for phosphate in seawater.



Appendix 4 (continued)

The optical density was measured between 10 minutes and 2 hours later in 1 cm glass cuvettes at 543 nm.

Distilled water blanks and standard ammonium solutions were carried out in a similar way with 1.0 ml of potassium bromide solution added to the water before the oxidising reagent to act as a catalyst.

Sample calculation

Seawater sample	OD = 0.2225
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Distilled H ₂ O blank	OD = 0.0950
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Corrected seawater sample	OD = 0.1275
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From the standard curve (Fig. Aiii) OD 0.1275

$$= 0.552 \mu\text{g-at N/l}$$

But this also includes the nitrite already present in the water sample. The concentration of ammonium in the seawater sample is

$$0.552 - 0.156$$

$$= \underline{0.396 \mu\text{g-at N/l}}$$

APPENDIX 5

WATER ANALYSIS - PHOSPHATE

Reagents:

- Ammonium molybdate solution (3.0 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 100 ml distilled water)
- Sulphuric acid solution (28 ml conc. H_2SO_4 in 180 ml distilled water)
- Ascorbic acid solution (27 g ascorbic acid in 500 ml distilled water)
- Potassium antimonyl tartrate solution (0.68 g $\text{KSbO}_3 \cdot \text{C}_4\text{H}_4\text{O}_6$ in 500 ml distilled water)
- Mixed Reagent (18 ml ammonium molybdate solution; 45 ml sulphuric acid solution; 18 ml ascorbic acid solution; 9 ml potassium antimonyl tartrate solution)

Method

5 ml of mixed reagent is added to a 50 ml seawater sample in a conical flask and the solution shaken. After 15 minutes at room temperature the optical density of the blue phosphomolybdate compound is measured in 10 cm glass cuvettes on a Corning Absorptiometer (red filter 609). Distilled water blanks and standard phosphate solutions were analysed in a similar way.

Appendix 5 (continued)

Sample calculation

Seawater sample	OD = 44.45
Reagent blank	OD = 10.73
Corrected seawater sample	OD = 33.72

Using the corrected seawater sample value, the phosphate concentration is read from the phosphate standard curve (Fig. Aiv)

Phosphate concentration of seawater sample is

1.738 $\mu\text{g-at P/l}$

APPENDIX 6

CARBOHYDRATE ANALYSES

1 MANNITOL

The tissue was extracted 3x in hot ETOH and one wash in cold ETOH. Washings were added to the total extract and made up to 25 ml with 80% ETOH. A 5 ml aliquot of the alcohol extract was evaporated to dryness; the volatile derivatives were prepared by redissolving the dried extract in the reaction mixture to give a final volume of 1.0 ml. 10 μ l of this was injected into the GLC (Fig. Av).

Sample calculation

Mannitol peak height = 52.75 units.

In order to relate this to the calibration curve (Fig Avi) this figure must be corrected to allow for the changing sensitivity of the GLC column during use. This variation was estimated by injecting a mannitol standard (1 ml/ml) every fifth run and using this new peak height in relation to the 2 sample runs on either side of it.

Original standard peak height = 33.1 units

New standard peak height = 38.5 units

$$\frac{\text{Sample peak ht} \times \text{original std (1 mg/ml) peak ht}^*}{\text{new std (1 mg/ml) peak height}}$$

* Used in construction of the calibration curve.

$$= \frac{52.75 \times 33.1}{38.5} = 45.35 \text{ units}$$

Fig. A v. GLC trace for mannitol and laminarin.

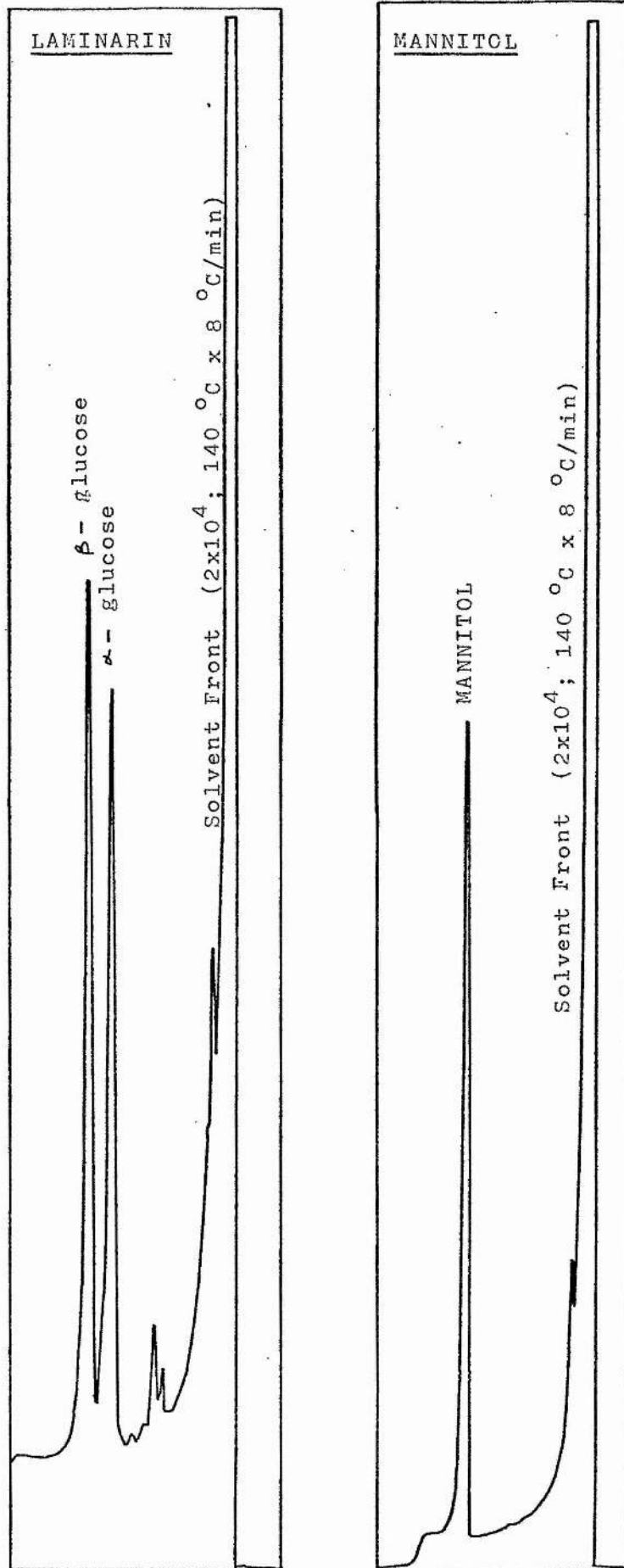


Fig A vi. Mannitol calibration curve
(Attenuation 2×10^4)

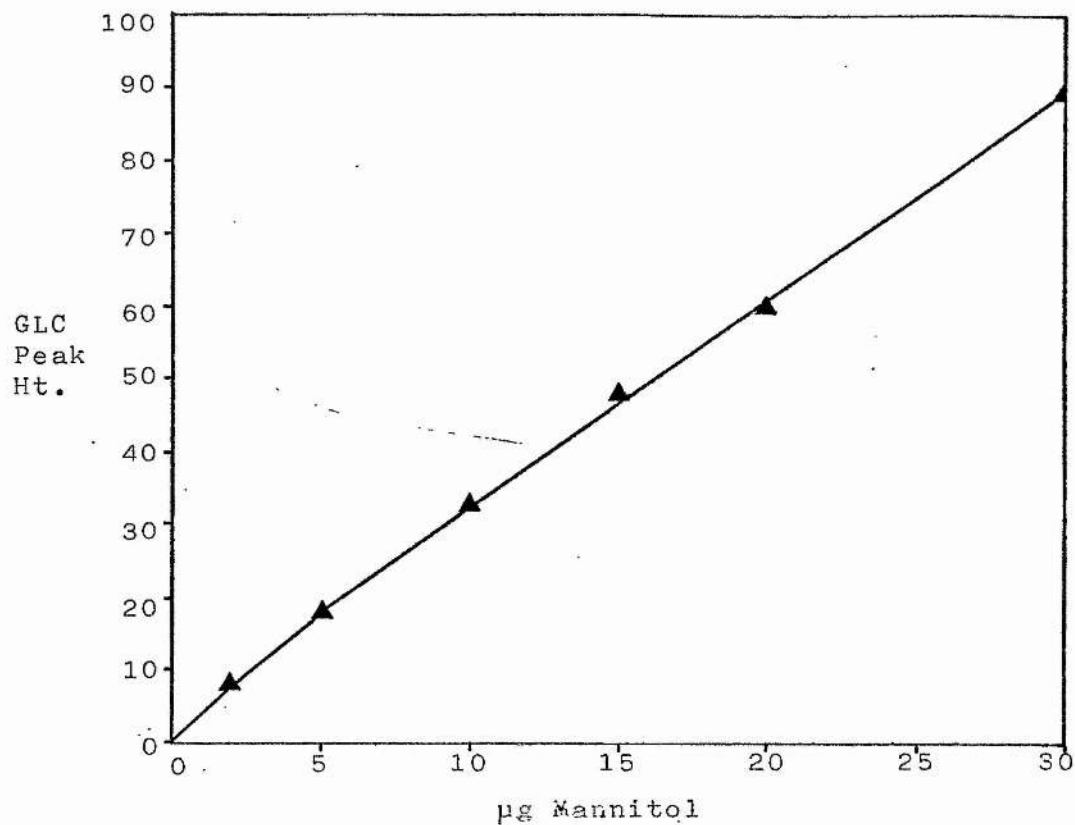
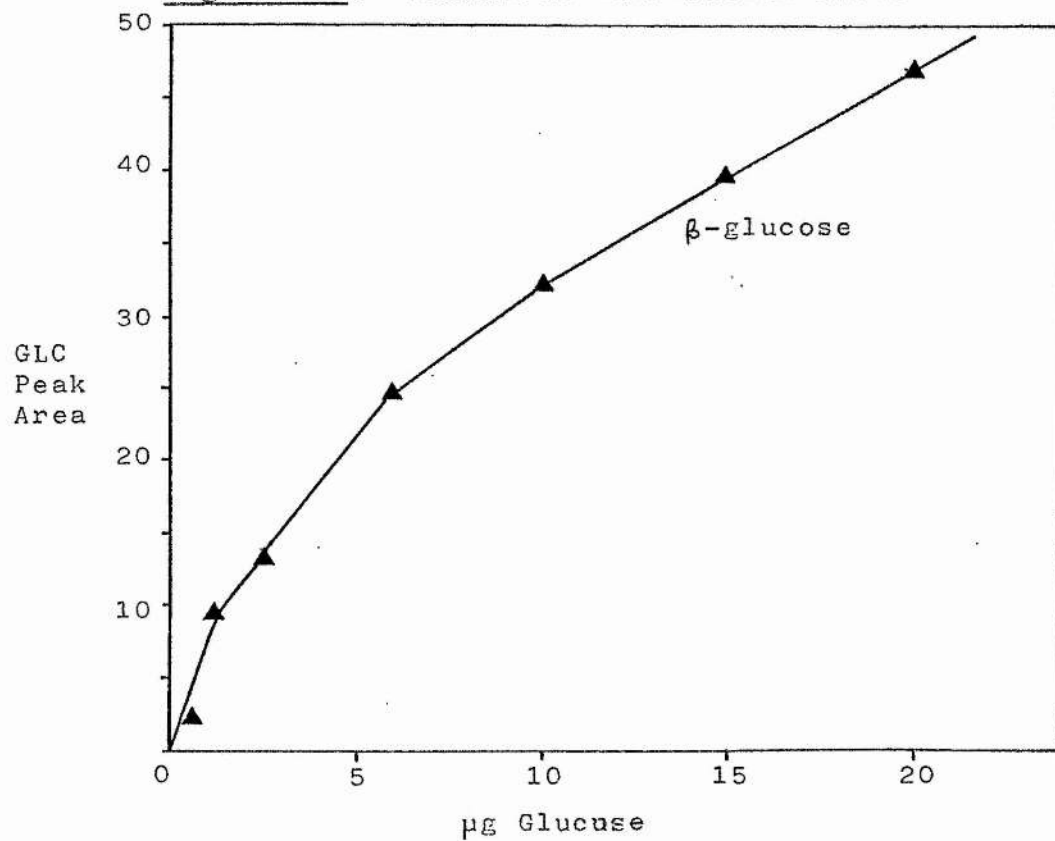


Fig. A vii. Laminarin Calibration curve



Appendix 6 (continued)

From the calibration curve

$$\begin{aligned}
 45.35 \text{ units} &= 14.5 \text{ } \mu\text{g mannitol injected} \\
 &= 1450 \text{ } \mu\text{g mannitol in the total} \\
 &\quad \text{reaction mixture} \\
 &= 7250 \text{ } \mu\text{g mannitol in the total} \\
 &\quad \text{alcohol extract}
 \end{aligned}$$

Dry wt of extracted tissue = 31.6 mg

Dry wt of ETOH extract = 17.0 mg

Dry wt of tissue 48.6 mg

7250 μg mannitol in 48.6 mg of tissue

= 14.92 mg mannitol/100 mg dry wt

ii LAMINARIN

The extracted dry tissue from the mannitol analysis was hydrolysed with 2.5 ml NH_2SO_4 for 6 hours in foil-capped test-tubes in a boiling water bath. The hydrolysate was decanted, the algal tissue was washed with distilled water, the hydrolysate and washings then made up to 10 ml with mixing. 2 ml of the hydrolysate solution were neutralised with barium carbonate powder. The solution was centrifuged for 10 minutes, 1 ml of the supernatant was removed and evaporated to dryness on a rotary evaporator. The dried extract was redissolved in 1.0 ml reaction mixture, 10 μl of this was injected into the GLC (Fig. Av).

Appendix 6 (continued)

Sample calculation

β -glucose peak height = 12.5 units

Width at half height = 1.5

Peak Area = 18.75 units

This must be related to the calibration curve in a similar way to the mannitol analysis (6i). The changing sensitivity of the GLC column was estimated by injecting a glucose standard (1.0 mg/ml) every fifth run and using this new peak height in relation to the 2 sample runs on either side of it.

Original standard β -glucose peak area = 32.24

New standard " " " = 40.25

Sample peak area x original std (1 mg/ml) peak area

New std (1 mg/ml) peak area

$$= \frac{18.75 \times 32.24}{40.25} = 15.02 \text{ units } \beta\text{-glucose}$$

From the calibration curve (Fig. A vii).

15.02 units \equiv 2.48 μ g β -glucose

The relationship between α - and β -glucose is constant

$$\frac{\alpha}{\beta} = 0.600$$

2.48 x 0.600 μ g α -glucose

= 1.49 μ g α -glucose

\equiv 3.97 μ g ($\alpha + \beta$) glucose \equiv 3.97 μ g laminarin

= 397 μ g laminarin in total reaction mixture

= 3970 μ g laminarin in total hydrolysate

Appendix 6 (continued)

Dry weight of tissue = 74.3 mg

3970 μ g laminarin in 74.3 mg dry wt of tissue

= 5.343 mg laminarin/100 mg dry wt

iii ALGINIC ACID

Method

10 ml of 0.2N sulphuric acid is added to 100 mg dried and ground frond tissue and left to stand overnight. The sample is filtered through a sintered glass crucible, washed with 5 ml of distilled water, the residue is washed into a beaker with 20 ml distilled water, 20 ml of 6% Na_2CO_3 is added and the mixture kept at 50 °C for 2 hours with occasional stirring, and then left overnight. The mixture is then filtered, washed with 20 ml 1.5% Na_2CO_3 followed by 3 x 10 ml washes with distilled water. The filtrate and washings are transferred to a tap funnel with a fine exit tube and run slowly through a fine jet (about 1 mm) into 30 ml of 10% calcium chloride solution with gently stirring. The solution is filtered; 20 ml N hydrochloric acid is added carefully (to remove the calcium ions). Gentle suction is applied to the filter and a further 20 ml N HCl is added. The alginic acid is then removed from the filter to a beaker, 50 ml distilled water and 30 ml N/2 calcium acetate solution are added. The

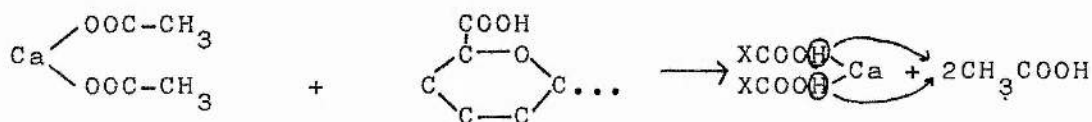
Appendix 6 (continued)

mixture is shaken and left to stand for 1 hour. The liberated acetic acid is then titrated with N/10 sodium hydroxide solution using phenolphthalein as the indicator.

Sample calculation

In the above reaction calcium acetate and mannuronic acid combine, releasing acetic acid (CH_3COOH). 1 gram molecule of alginic acid results in 1 gram molecule of acetic acid.

Calcium acetate + Mannuronic acid



(X = mannuronic acid)

1.5 ml NaOH (N/10) is used to neutralise the solution

$$\frac{1}{10} \times \frac{1.5}{1000} \text{ Gram molecules acetic acid (} \underline{=} \text{ GM alginic acid)}$$

Alginic Acid ($\text{C}_6\text{H}_{12}\text{O}_6$) mw = 176 g

$$\frac{1.5 \times 176}{10,000} \text{ g alginic acid}$$

= 0.0264 g extracted from 100 mg tissue

Alginic acid content is 26.4% dry wt

APPENDIX 7

TISSUE NITROGEN ANALYSIS

Discs of tissue (2.5 cm diameter) cut from the frond were analysed for inorganic (NO_3^-) and organic nitrogen.

The disc was cut into small pieces, extracted 3 x in hot 80% ETOH, followed by one wash in absolute ETOH and 2 washes in diethyl ether. The combined extracts were made up to a volume of 25 ml. The insoluble material was dried to obtain the extracted dry weight. 5 ml of the alcohol extract was dried and weighed (x5) to give the dry weight of the ethanol-soluble extract.

Inorganic Nitrogen (NO_3) was analysed by evaporating 5 ml of the ethanol-soluble fraction to dryness, redissolving the salts in 100 ml distilled water and determining reactive NO_3 as in Appendix 3.

Sample calculation

Extracted dry wt of tissue	60.6 mg
Dry wt of ETOH-extract	<u>27.0 mg</u>
Dry wt of tissue	87.6 mg
Sample $\text{OD}_{543} = 0.449$	$= 8.685 \mu\text{g-at N/l (Fig. Aii)}$
	$= 12.159 \mu\text{g N in 100 ml}$
	$= 60.795 \mu\text{g N in total extract}$
	$= 60.795 \mu\text{g N in 87.6 mg dry}$
	wt of tissue
	$= \underline{69.401 \mu\text{g N/100 mg dry wt}}$

Organic-N

Extracted Dry Tissue. Although Kjeldahl digestion

Appendix 7 (continued)

was complete using all the extracted dry tissue from the whole disc, analysis of ammonia was saturated when this amount of tissue was used. It was found that after digestion and neutralisation (Strickland & Parsens 1968) analysing 10% of the extract diluted to 50 ml with distilled water for ammonia analysis (Appendix 4) gave reproducible results.

The ammonia is oxidised to nitrite by the assay described above. The optical density of the sample is read in 1 cm glass cuvettes at 543 nm.

Sample calculation

Dry wt of tissue (as above) = 87.6 mg

$OD_{543} = 0.428 = 30.0 \mu\text{g N}$ from the standard curve

(Fig. Aviii)

$= 300 \mu\text{g N}$ in total extract

$= 300 \mu\text{g N}$ in 87.6 mg dry wt tissue

$= \underline{342.47 \mu\text{g N/100 mg dry wt}}$

Organic-N

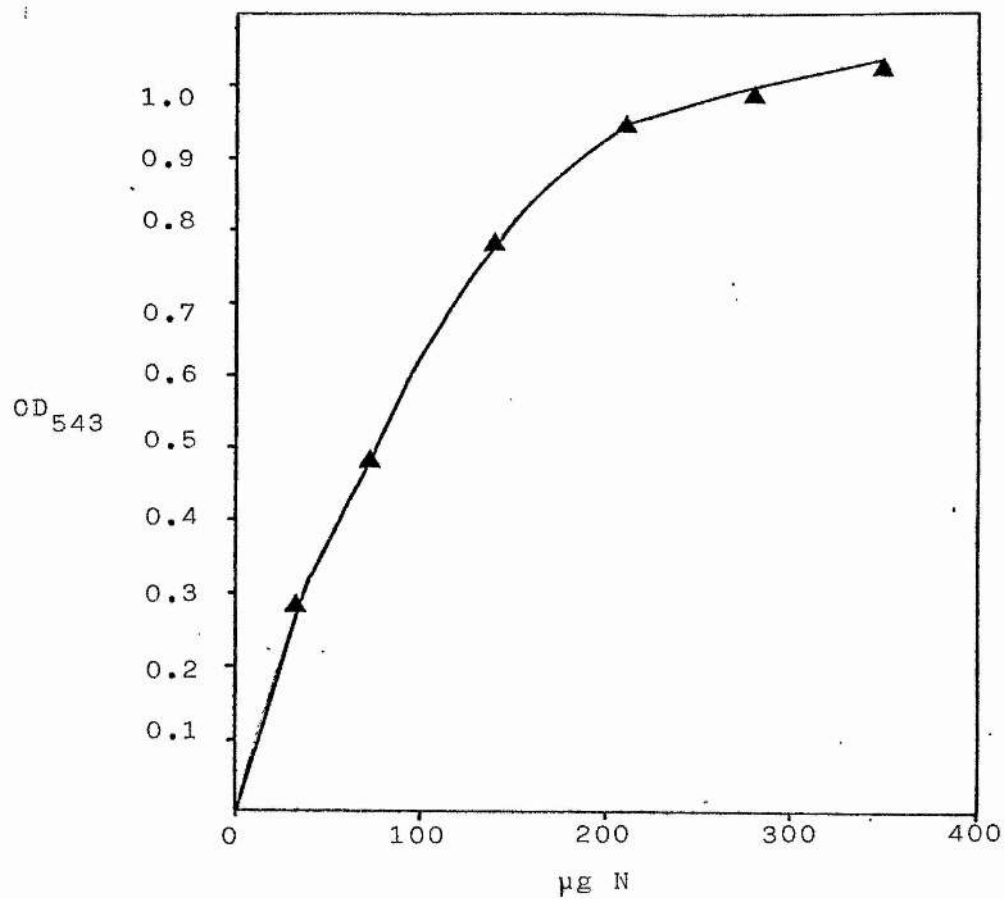
Ethanol Extract. The organic-N in 5 ml of the ethanol extract was converted to ammonia by Kjeldahl digestion. After digestion and neutralisation the extract was diluted with distilled water to give a volume of 50 ml, for ammonia analysis (Appendix 4).

Sample calculation

Dry wt of tissue (as above) = 87.6

$OD_{543} = 0.551 = 41.0 \mu\text{g N}$ from standard curve (Fig. Aviii)

Fig. A viii. $\mu\text{g N}$ in sample after Kjeldahl digestion of organic-N to ammonia followed by oxidation to nitrite.



Appendix 7 (continued)

\equiv 205.00 $\mu\text{g N}$ in 87.6 mg dry wt tissue

\equiv 234.02 $\mu\text{g N}/100$ mg dry wt

Total N in the tissue is the sum of the 3 fractions.

Inorganic-N	69.40
Protein-N	342.47
Non-protein-N	<u>234.02</u>
Total-N	<u>645.89 $\mu\text{g N}/100$ mg dry wt</u>

APPENDIX 8

TISSUE PHOSPHATE ANALYSIS

A disc of tissue cut from the frond of L. digitata or L. saccharina was oven dried, ground and hydrolysed in 2.5 ml N H₂SO₄ in a boiling water bath for 4 hours. 0.1 ml of the hydrolysate was diluted to 200 ml with distilled water and analysed for reactive phosphate (see phosphate water analysis, appendix 5)

Sample calculation

Tissue sample	OD = 55.0
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Reagent blank	OD = 9.9
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Corrected tissue sample	OD = 45.1
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From the phosphate standard curve (Fig. Aiv)

$$\text{OD } 45.1 = 2.4 \text{ } \mu\text{g-at P/l}$$

In 200 ml there are 0.48 $\mu\text{g-at P}$ or 15.36 $\mu\text{g P}$

= 384 $\mu\text{g P}$ in total hydrolysate

Dry wt of disc = 113.5 mg

Relative tissue phosphate is

$$\underline{338.33 \text{ } \mu\text{g P/100 mg dry wt}}$$